

Effect of *Curcuma Longa* (Turmeric) on Postprandial Glycemia in Healthy, Non-diabetic

Adults

by

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ABSTRACT

Curcumin is an active ingredient of *Curcuma longa* (Turmeric) and is studied extensively for its antioxidant, anti-inflammatory, anti-bacterial, anti-viral, and anti-cancer properties. The purpose of this study was to examine the effects of turmeric on blood glucose and plasma insulin levels. The study utilized a placebo-controlled, randomized cross-over design with participants serving as their own control. Eight glucose tolerant healthy participants completed the full study. Three-weeks washout period was kept in between six-weeks. Prior to the test meal day, participants were asked to eat a bagel with their evening dinner. During the day of the test meal, participants reported to the test site in a rested and fasted state. Participants completed mashed potato meal tests with 500 mg of turmeric powder or placebo mixed in water, followed by 3 weeks of 500 mg turmeric or placebo supplement ingestion at home. During this visit blood glucose fingerpicks were obtained at fasting, 30, 60, 90, and 120 min post-meal. Blood plasma insulin at fasting and at 30 min after the test meal were also obtained. During week 4, participants reported to the test site in a rested and fasted state where fasting blood glucose finger pricks and blood plasma insulin were measured. During week 5 to 7, participants were given a washout time-period. During week 8, entire process from week 1 to 4 was repeated by interchanging the groups. Compared to placebo, reduction in postprandial blood glucose and insulin response were non-significant after ingestion of turmeric powder. Taking turmeric for 3 weeks had no change in blood glucose and insulin levels. However, taking turmeric powder supplements for 3 weeks, showed a 4.4% reduction in blood glucose. Change in insulin at

30 min were compared with baseline insulin level showing no significant change between placebo and turmeric group. Fasting insulin after 3-weeks consumption of turmeric did not show any significant change, but showed a larger effect size (0.08). Future research is essential to examine the turmeric powder supplement benefits over a long period of time in healthy adults and whether it is beneficial in preventing the occurrence of type 2 diabetes.

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CHAPTER 1

INTRODUCTON

The International Diabetes Federation estimates that the number of diabetes cases will rise to 592 million by the year 2035 as compared to 387 million in 2015, a 53% increase (International Diabetes Federation,2015). There are 4.9 million deaths annually due to diabetes or one death every seven seconds, one person dies from it. (International. Diabetes. Federation. 2015). It has shown that one of the biggest contributor to diabetes is the lifestyle, including diet that mainly consists of complex carbohydrate, especially high in glycemic index (Maki et.al. 2015). In addition, positive association has seen between red meat consumption and diabetes (Benedinelli et.al, 2012). In type 2 diabetes, a decrease in insulin sensitivity may be due to decrease in a beta-pancreatic cells function causing a defect in insulin secretion (Porte et.al 2001). According to Gandhi (2014), diabetes is increasing in every country due to obesity and unhealthy lifestyles. However, it has also observed that insulin resistance can also occur in non-obese individual and aids in chronic diseases like cardiovascular, atherosclerotic (Hekmatdoost A, 2011).

Curcuma longa (turmeric) is a plant root and is a member of *Zingiberace* family. It is widely grown and consumed in India and Asian countries in the form of spice to flavor and give color to food. It is an ancient medicine used in Ayurveda and as a home remedy for cough, cold and several diseases. It is also used as a coloring agent in the form of yellow-orange dye E100 in cheese, yogurt, and other food products (Esatbeyoglu T, 2012). There is a research supporting the medicinal effect of turmeric in curing several diseases related to inflammation, stomach ailments and infection (Aggarwal, 2007). Due

to its anti-inflammatory and antioxidant mechanism, this polyphenol has been studied in life-threatening diseases like cancer and Alzheimer's (Esatbeyoglu, 2012).

C.longa has three active ingredients viz. curcuminoids, demethoxycurcumin and bis-demethoxycucrcumin. A number of studies have examined the active ingredient curcuminoids decrease in blood glucose and increase insulin sensitivity in type 2 diabetes. It has been suggested that curcuminoids supplementation decreases FFA's (Free Fatty Acids), promoting fatty acid oxidation and utilization. This causes glucose to decrease in the body (Na LX, 2012). Other studies also state that curcuminoids has an effect on oxidative stress by increasing antioxidant enzymes (Yang W et.al, 2015).

Turmeric ingestion is considered safe; however, individuals with gall bladder, or liver diseases are advised not to ingest turmeric as a dietary supplement (NCCIH). Oxalate in turmeric is easily soluble in the body, and kidney stones are 80% made up of oxalate. Hence, people who are prone to kidney stones or have a history of kidney stones are advised not to take oxalate over 50 mg a day. It has been observed in animal studies that *C.longa* helps to lower blood glucose significantly in diabetic albino rats (N A, 2002). It has also been observed that when turmeric supplementation is given with an anti-hyperglycemic therapy like metformin, molecular complications of type 2 diabetes can be prevented (Salvi NM et.al, 2015). Recent research suggests that turmeric is a potent inhibitor of pancreatic α -amylase, a digestive enzyme that hydrolyzes starch to basic glucose units that are absorbed in the intestinal tract and released in the portal blood (Ponnusamy S, 2012). Thus far only one study has reported directly examining the effect of turmeric ingestion on healthy individuals measuring postprandial blood glucose. Due

to lack of proper protocol, their results did not suggest that turmeric effects in altering postprandial glucose in healthy adults (J Wickenberg, 2010). The researchers have used Glucola as a means of high glycemia. Thus, more study is required to see the effect of turmeric ingestion in healthy adults on the postprandial blood glucose.

Purpose of Study

The purpose of this experiment is to compare the effect of turmeric on postprandial glycemia in healthy glucose tolerant subjects following an ingestion of a high glycemic carbohydrate food.

Research Aim

This study will investigate the impact of 500 mg of turmeric ingestion in the form of dried root powder on postprandial glucose and insulin concentrations in healthy human subjects.

Hypothesis

Turmeric, 500 mg as a dried root powder as compared to placebo will significantly reduce the 2-hour postprandial glycemic response after following a test meal containing 80 g of starch in the form of mashed potato in healthy non-diabetic adults.

Definition of Terms

- Acarbose®: An oral medicine used in treatment of Type 2 diabetes where it blocks the enzyme that digest starch in food (diabetes.org).
- Alpha-glucosidase inhibitors: class of drugs used in the treatment of Type 2 diabetes where it blocks the enzyme that digest starch in food. Migitol® and Acarbose® are the generic names of the drugs used in this class (diabetes.org).
- Beta-cell: a cell that makes insulin and is located at the islets of pancreas.
- Bioavailability: the degree to which a substance or a drug is absorbed into the physiological system and is made available at the activity site.
- *Curcuma longa*: a perennial plant belonging to Zingiberace family, where its rhizome is used as turmeric (Ponnusamy S, Zinjarde s, et.al. Food Chemistry, 2012)
- Fasting insulin ≥ 18 mU/mL (Mosby's lab manual)
 - Fasting plasma glucose: 70-115 mg/dL
- Glucose ranges: (Mosby's lab manual)
 - 120 min postprandial: 70-140 mg/dL
 - 180 min postprandial: 70-115 mg/dL
 - 30 min postprandial: 110-190 mg/dL
 - 60 min postprandial: 120-190 mg/dL
 - 90 min postprandial: 95-165 mg/dL
- Insulin Resistance: An impaired tissue response to the action of insulin

- Postprandial plasma glucose: the glucose area-under-the-curve for the 2-hour period following meal consumption

Delimitations

This study enrolled glucose tolerant healthy weight stable participants, not following a vegetarian diet, and are not taking turmeric supplements.

Assumptions

It was assumed that participants complied with all pre-testing diet and physical activity protocols:

- consumed provided bagel with dinner meal in its entirety prior to coming for the meal testing
- refrained from exercise, as well as intake of caffeine and alcohol for 24 hrs prior to data collection
- appropriately fasted on the morning of the trial
- and, consumed provided supplements for 21 days with meal

Limitations

This research is limited by participant adherence to protocol. Sample size may have limited the data interpretations. Turmeric dosage of 500 mg may also have played a role in the study limitation.

CHAPTER 2

REVIEW OF LIETERATURE

History of *Curcuma Longa* (turmeric)

Curcuma longa (turmeric) is a plant that is mostly grown in Asia, India and China. It is a plant root that belongs to the ginger family Zingiberaceae. In the 13th century, Arabian merchants brought turmeric to the European market from India, where it got the name “Indian saffron” (Aggarwal 2007). During 15th century it was combined with other spices to form curry powder. The plant grows in a hot, humid climate and it requires a large quantity of water. It grows up to one-meter-long, has oblong, tufted leaves and produces 6-10 tubers (Wickenburg 2010). The tubers are dried and then grounded to get the golden-yellow turmeric powder.

Turmeric and Its Components

Turmeric has main three components: Curcuminoids, dethoxycurcumin and bisdemethoxycurcumin. Curcuminoids contain curcumin and is known to have the highest bioactive component that consists of 3 to 5% in a turmeric root. It is very rare to find pure curcumin, however it is possible to get the three main components of turmeric from the mixture of turmeric power by following crystallization and column chromatography method (Osorio-Tobon JF 2016). Due to curcuminoids in rhizome, turmeric gets its golden-yellow color. Turmeric contains 1-5% of an essential oil that is used in aromatherapy (Estabeyoglu 2012). Curcumin is insoluble in water and has a

lipophilic property (Jurenka J 2009). It is most stable from 1.0 pH to 6.0 pH, found mostly in the stomach and intestine. (Shashikumar 2005).

Turmeric Uses

Turmeric is primarily used as a spice in Indian and Asian cuisine that gives food a distinct flavor and golden color. It has been used in Ayurvedic medicine as well as other eastern medicines for at least 2500 years (Gupta 2013). Its medicinal properties have been used for inflammatory disorders, arthritis, stomach ailments, sores, infections, cough, cold, acne, and burn etc. (Ghorbani 2014). There has been no reliable data to support these medicinal usages of turmeric due to few clinical trials (NCCIH 2012). Turmeric is also used to make color. It makes the orange-yellow dye E100 to use in cheese, butter, mustard, dairy products and canned fish (NCCIH 2012). Due to its bitter taste and piquant earthy scent it is not used to season desserts and cakes (Estabeyoglu 2012).

Bioavailability

The bioavailability of curcumin is primarily dependent on the metabolism within the body, especially in intestine and liver. Oral bioavailability of curcumin is low in the body due to less small intestine absorption. This has been seen in a study done on rats, where 1 g/kg body weight of curcumin was given orally and most of it got excreted through feces. (Suresh 2010, Ravindranath 1980, Estabeyoglu 2012, Shoba 1998).

Additionally, the liver has a very high bioavailability. Only high doses of curcumin in plasma is bioavailable and it has been observed within the first two hours of ingestion. Curcumin is metabolized by Phase I and II enzymes like cytochrome P450 monooxygenase, alcohol dehydrogenase, methyltransferase, acetyl co-enzyme A etc. It has been noted that curcumin inhibits phase I metabolism while it stimulates phase II metabolism. In phase II metabolism of curcumin, they are conjugated with glucuronic acid and sulfate present in enterocytes and hepatocytes (Hoehle 2007). These curcumin conjugates exit the enterocytes with the help of multidrug resistance-related proteins (MRP) namely MRP1 and MRP2 respectively. These are under the influence of transcriptional factor known as nuclear factor 2 (Nrf2) that gets turned on by curcumin (Estabeyoglu 2012). This shows that curcumin has a poor bioavailability in the body and is excreted easily once ingested.

It is known that curcumin is lipophilic and insoluble in water. Hence, in order to increase the bioavailability, research has shown that when curcumin is taken with piperine it can increase the bioavailability by two folds. Piperine is a molecule present in pepper vine, piper nigrum, hot jalapeno, peppers and peppercorns (Sharma RA, 2005). In one study, when humans were given 2 grams of curcumin along with 5 grams of piperine, it showed that in two hours, the bioavailability increased by two fold when compared with only curcumin consumption subjects (Arcaro 2014). In another study by Shoba and colleagues when 2 g of curcumin was administered with 20 mg of pepper constituent in fasting individuals it was observed that bioavailability of curcumin increased by 2000% (Shoba G, et al., 1998). It has been understood by the researchers

that in the presence of piperine, glucouronidation of curcumin gets inhibited. Thus, there is no Phase II metabolism of curcumin where it can no longer go through conjugation reactions. In the presence of piperine, curcumin cannot further convert into a polar, water soluble form and it cannot excrete through renal system (Srinivasan 2010). Hence, piperine can help in increasing curcumin bioavailability.

Safety of Turmeric

Turmeric has been considered safe for use in the form of spice for centuries. As of now, there has been no data registered regarding the toxic effects of turmeric in a general population. (NCCIH 2012, Lao et.al 2006). The U.S. Food and Drug Administration (FDA) has accepted turmeric as a safe ingredient even in high doses. However, Tang (2008) finds that due to the presence of oxalates in turmeric, people who have history of kidney stones or are at high risk of developing one, should not take turmeric. The study was a randomized crossover where six subjects were signed for 3.0 g of cinnamon treatment and 5.0 grams for turmeric treatment for a four weeks' period. Subjects were required to take the respective two supplements three times a day. Subjects were given breakfast containing low calcium and less oxalate. Oxalate, creatine and calcium content were measured from 6 and 22-hour urine analysis. It was noted that turmeric had a high oxalate content of 91% and cinnamon had only 6%. This shows that consumption of turmeric can significantly increase urinary oxalate levels and can increase the risk of kidney stone formation.

Turmeric and Scientific Research

Turmeric has been one of the most studied herbs in the modern world. The interest has increased in studying this herbal plant especially due to its therapeutic importance. In addition, current research trend shows that there is an increase in polyphenol studies due to their protective roles against various chronic diseases like cardiovascular and cancer. Curcumin is the most active component of turmeric. Despite its traditional use, several studies mainly in pharmacology have measured its medicinal properties like antioxidant, anti-inflammatory, anti-bacterial, anti-tumor etc. Curcumin has these multiple effects due to its polyphenol property and can modulate several physiological pathways (Gupta 2013). It is understood that there are several researchers sited related to the turmeric's active ingredients and compounds.

In vivo, it has shown that curcumin, an active ingredient of turmeric decreases the activation of human dendritic cells when inflammatory cytokines are activated (Karsofsky 2009). This shows that turmeric extracts have an anti-inflammatory effect. Besides this, turmeric has a positive effect on myocardium injury. In a study, when Wistar albino rats were fed 100 mg per kg turmeric daily for one month, anti-apoptotic activity was noticed showing a cardio protection of turmeric. This increased the functional recovery period related to cell necrosis (Mohanty et. al 2006).

Several studies have described anti-carcinogenic and chemo protective effects of curcumin. These preventive effects of curcumin are directly or indirectly regulated by signal transduction pathway. Tumor suppressor gene p53 has been known for important cell growth and apoptosis. When the cells in the body get dysregulated, p53 pathway in

the presence of curcumin takes over and helps in DNA-binding activity leading it to apoptosis of the cell. Curcumin can also lower the p53 gene expression in non-malignant cells. Thus, curcumin can prompt cancer killing cells with the p53 pathway and act as a chemo protective agent (Sa 2008).

Curcumin consists of antioxidant, anti-inflammatory and lipophilic properties. Due to the presence of these properties and observing an association between turmeric consumption and lower incidence of Alzheimer's disease in Indian and Asian population by 4.4 times, there is a growing evidence that curcumin consumption can help in Alzheimer disease including dementia (Ganguli M et al., 2000). In a study, association of turmeric powder in the form of curry in 1010 Asians, aged between 60 and 93 years was investigated. It was noted that individuals who ate curry more than once a month had better perform cognitive test than who ate curry less than a month (Ng TP., 2006). According to World Health Organization (WHO), 5% men and 6% women above the age of 60 years are affected with Alzheimer's type dementia worldwide (Fratiglioni L., et al, 1999). In pathogenesis of Alzheimer's disease there is a chronic inflammation of nerve cells. Due to anti-oxidant property present in curcumin, it binds to certain proteins in monocytes and suppress gene factor (Erg-1) and reduces inflammation (Pendurthi UR, Rao LV., 2000). It has also understood that in the presence of curcumin, pro-inflammatory cytokines (IL-1, IL-6, and TNF) production is inhibited (Mishra S. et al., 2008). This suggests that, curcumin works as a potent inhibitor of pro-inflammatory and cytokine production. Curcumin's antioxidant property was studied on rats and it was observed that when curcumin is administered, lipid peroxidation and lipofuscin levels,

products of brain damage decreased significantly (Bala K et al., 2006). It has also been studied that in Alzheimer's disease there is a huge accumulation of plaques known as beta-amyloid plaques (Mishra S. et al., 2008). In vitro, when these plaques present in macrophages were treated with curcumin it was observed that the plaques decreased significantly (Zhang L., et al., 2006). This suggests that curcumin has a beneficiary effect in Alzheimer's disease.

Turmeric when taken as a dietary supplement can increase the pancreatic lipase chymotrypsin along with amylase activity. In addition, when turmeric is mixed with other spices like black pepper and cumin it has shown an increase in bile flow and secretion (Plate 2002). Thus, turmeric can act as a digestive stimulant.

Concurrently, turmeric has been studied in humans to see the counter effect against several diseases. Bundy and colleagues (2004) studied the effects of turmeric on irritable bowel syndrome in randomized partially blinded pilot study in 207 volunteers. It was observed among volunteers that 53% reported their bowel changed when they took 72 mg of one tablet daily and 60% reported with their bowel changed after taking 2 tablet daily. In addition, it was noted that there was a decrease in volunteer's abdominal pain and discomfort levels by 22% and 25% in the one- and two-tablet groups respectively (Boudy R, Walker AF, Middleton RW, Booth J., 2004). Hence, turmeric may help in reducing irritable bowel syndrome symptomology.

Turmeric supplementation has shown a healing effect in peptic ulcers. Prucksunand and colleges (2001) did a clinical trial study where 45 patients with peptic ulcer received 300 mg, 2 turmeric capsules, five times a day. After four weeks of the

treatment it was noted that peptic ulcer was cured by 48% in the patients, which further increased to 76% at the end of 12th week. Besides this, Shimouchi et al. (2009) studied in eight healthy subjects that 500 mg of turmeric taken in the form of curry with rice increases hydrogen producing bacterial flora in colon and bowel motility after 12 hours of fasting. This shows that dietary turmeric can activate bowel motility along with carbohydrate colonic fermentation.

Besides turmeric supplementation, turmeric oil has also been studied in various conditions. Honda et al. analyzed the hepatic gene expression in mice when turmeric oil containing 14% by weight curcuminoids (6.5% curcumin, 2.3% demethoxycurcumin and 1.9% bisdemethoxycurcumin) was ingested in obese diabetic mouse for one week. This study was done by using DNA microarray analysis, a polymerase chain reaction (PCR) by comparing different oils. It was observed that turmeric oil inhibited the increase of blood glucose levels in the diabetic mice along with abdominal fat (Honda S, Aoki F., Tanaka H et al., 2006).

Turmeric and Glucose Metabolism

Several studies have examined the effects of curcumin in blood glucose levels. A study conducted by Arun N and Nalini N (2002) measured the efficacy of turmeric on blood sugar in diabetic albino rats. During the study, diabetic albino rats were administered with 80 mg/kg with curcumin for 21 days. This showed a significant decrease in blood sugar, hemoglobin, and glycosylated hemoglobin. Further analysis

showed that curcumin also decreased the sorbitol dehydrogenase enzyme activity that further converts sorbitol to fructose. In another study it was shown that when 10, 20 and 30 mg/kg body weight of turmeric extract was given orally to streptozotocin (STZ) induced rats for 42 days, it showed a decrease in blood glucose levels. The best results of decreased blood glucose were measured when turmeric was administered at 30 mg/kg body weight.

Anti-glycemic effects have also been examined in rats. In a research study done by Weisberg SP et al. 2008, obese male rats and diet induced male rats have been studied. After two weeks of the treatment, 3% dietary curcumin decreased the blood glucose levels. It also showed decrease in the 2-hour glucose tolerance test results where 3% by weight of curcumin was given along with 4% fat by weight or high fat diet containing 35% fat for two weeks. This also shows that curcumin decreased the HbA1c levels in both diet induced mice as well as obese mice after 5 weeks of curcumin treatment. In addition, insulin resistance was also measured in this study, where both the types of rats were kept in a cage for 6 hours and every fifteen minutes, blood glucose levels were measured for a total of 2 hours. The results showed a decrease in area under the curve of the obese male rats' percent basal glucose.

The effects of curcumin on glycemia in diabetic models of rats and mice have been explained by El-Azab his study. When 10 mM curcumin was injected for 28 days it decreased the tumor necrosis factor- α (TNF- α) levels along with IL-1B (pancreatic islets) due to its anti-inflammatory properties. In another study it was noticed that plasma TNF- α level and free fatty acid levels, were directly correlated to obesity-related insulin

resistance and T2DM. Thus, when curcumin was fed in rats, it improved the glucose tolerance when compared with the untreated rats. (El-Moselhy MA 2007). In addition, curcumin also can elevate plasma insulin levels and increase lipoprotein lipase (LPL) activity (Sea I-K 2008). This was observed in a study to measure the effect of curcumin when male diabetic mice were fed curcumin for six weeks and then compared to non-diabetic male mice. The results showed that there was an increase in insulin resistance and glucose tolerance along with high insulin levels in diabetic mice who consumed curcumin 0.02% weight per body weight. Hence, it illustrates that curcumin increases the enzyme activity in liver that process glycolysis along with glycolysis and gluconeogenesis (Sea KI et al. 2008).

Interestingly, turmeric has been studied with other substances to see the combined effect in the glucose levels. Patumraj S. and colleagues (2006) studied the combined effect of vitamin C and curcumin in hyperglycemic Wistar Furth rats with hyperlipidemia. These streptozotocin induced rats were given 300 mg/kg body weight of curcumin along with 1 g/l of ascorbic acid mixed in drinking water for seven weeks. It was observed that blood glucose, lipid profiles, glycosylated hemoglobin were significantly decreased when compared to the control group of diabetic rats. Hence, this suggests that curcumin can enhance ascorbic acid metabolism in the body by protecting the endothelial cells due to its anti-oxidant property (Patumraj S., Wongeakin N., Sridulyakul P., Futrakul N., Bunnag S., 2006).

Gultierres and colleagues (2012) examined the prolonged effects of forty-eight diabetic rats to understand the changes that occurred with curcumin-supplementation

mixed in yogurt. Curcumin was mixed in the yogurt with different dose levels of 30, 60 and 90 mg/kg body weight per day. These various sample doses were given to diabetic rats and were compared with non-diabetic rats for 31 days. It was observed from the blood samples that rats who ate 90mg/kg body weight a day of curcumin mixed with yogurt decreased their glucose levels by 10 mmol/l and were noted to be steady at that rate. However, rats in control group showed no decrease in blood glucose levels. Additionally, urine samples were also collected and at the seventh day of the study, where rats with curcumin and yogurt showed a decrease in urine glucose levels when compared to the first day of urine samples. This fall in glycemia is furthermore explained by studying the pancreatic islets that curcumin could have stimulated the secretion of insulin in the body. Thus, this study suggests that the β -cells that got destroyed due to the fall of insulin levels in the body was due to curcumin probably altering the enzyme action and this could have helped in bringing glucose levels to a normal level.

It has been understood that in the presence of oxidative stress due to hyperglycemia, β cells generation slows down. A study was done in human pancreatic islets at a cellular level to see the effects of curcumin and its analogs on the induction of antioxidant enzymes in the β -cells of pancreas. With the help of double immunofluorescent staining techniques, islets were induced with curcumin and its different analogs in β -cells. This showed that there was an increase in the antioxidant enzymes like heme oxygenase at the cellular level in particular with the m-RNA and protein levels. Modularly sub-unit of gamma-glutamyl-cysteine ligase, a content of glutathione was increased along with basal insulin secretion. Thus, this cellular

mechanism of curcumin can suggest that it can help protect pancreatic islets from getting damaged due to oxidative stress. (Balamurugan 2009).

Furthermore, other analogs of curcumin have also showed antidiabetic properties. Bis-o-hydroxycinnamoylmethane, a natural curcuminoids of BDMC has been studied in diabetic rats and have shown positive results by increasing antioxidant defense system. (Srinivasan 2003). 0.05, 0.1, and 0.2 mmol/kg/day per body of bis (curcumino) oxovandaium has also shown a decrease in blood glucose levels in diabetic rats (Majithiya 2005).

In addition to decreased glucose absorption rate, curcumin has also been studied and shown to increase GLUT 4, GLUT2 and GLUT 3 gene expression. GLUT 2 and GLUT 3 are facilitated glucose transporters that carriers' glucoses across cell membranes and GLUT 4 is located on the cell membrane of muscle cells and adipocytes and is relied on insulin (Jellinger 2007). Insulin receptors bind to the adipocyte and muscle cell receptor creating a cascade that leads to GLUT-4 to plasma membrane. This creates an influx of glucose in the presence of insulin. On the contrary, when insulin is absent GLUT-4 in cytosol is surrounded by membrane vesicles.

In a study done by Peeyush et al. (2009), it was observed that curcumin can help mediate other neuroprotective roles in the presence of hyperglycemia. When 60 mg/kg curcumin was fed for 14 days to diabetic induced Wistar rats, GLUT 3 with gene expression of acetylcholine esterase, an enzyme that functions as neurotransmitters was observed. It was seen that the esterase enzyme increased GLUT 3, Muscarinic M1, M3, $\alpha 7$ nicotinic acetylcholine and insulin receptors in the cerebellum of diabetic rats. This

was noted when the insulin along with curcumin inhibited the enzyme action of acetylcholine esterase, GLUT 3, insulin and receptors. This shows that curcumin regulates the activity of cholinergic and insulin receptors respectively. Thus, this study shows that curcumin has a therapeutic role for protection along with prevention of aggressive diabetic complications (Peeyush KT., Gireesh G, Jobin M, Paulose CS., 2009).

Pugazhenthii and colleagues showed that the if further purification of curcumin and other analogy like demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) is done, then the expression of heme oxygenase-1 expression (HO-1) through PI3K/Akt signaling of β -cells in mice is regulated. Real-time reverse transcription polymerase chain reaction also showed that DMC and BDMC increased the levels of glutamyl cysteine ligase enzyme with the synthesis of glutathione and NADPH (Pugazhenthii S, Akhov L., Selvaraj G., Wang M, Alam J., 2007).

Turmeric in Human Studies

Turmeric has been seen to give a favorable effect on decreasing inflammatory cytokines and markers of oxidative stress in type 2 diabetes mellitus patients when compared with atorvastatin and placebo treatments. It is known that one of the clinical signs of atherosclerosis is the vascular complications and endothelial dysfunction. In the study conducted by Ushrani and colleagues, a formulated curcuminoids two capsules of 150 mg were given twice daily for 8 weeks. This was compared with the group of

participants who were taking 10 mg of atorvastatin, cholesterol-lowering medication and placebo one capsule twice daily. It was noted that curcuminoids formulated drug had a beneficial effect on endothelial functions along with decrease in inflammatory cytokines and malondialdehyde, however, total cholesterol and triglycerides did not change. As it is known from previous studies, atorvastatin significantly improved and decreased all of the factors including total cholesterol and triglycerides in type 2 diabetic patients. Thus, curcuminoids can have a beneficial role in protecting patients with high levels of cardiovascular risks by improving endothelia function (Usharani P, Mateen A, Naidu MU, Raju YS, Chandra N).

Na LX and colleagues studied the effect of curcuminoids for three months in 100 obese type 2 diabetic patients. 300 mg per day of curcuminoids supplements were given to the patients for 3 months. It was observed that patients taking 300 mg per day of curcuminoids, fasting blood glucose and insulin resistance index (Homeostasis Model Assessment of Insulin Resistance -HOMA-IR) reduced significantly along with HbA1c and free fatty acids (FFAs). This reduction is speculated due to decrease in FFAs which may result from promoting fatty acid oxidation and utilization (Na LX et al., 2012).

Proposed Mechanism

It has been observed that the principle bioactive component in curcumin is responsible for the inhibition of pancreatic α -amylase enzyme. It is known that alpha-amylase hydrolyses starch and glycogen. Now, when the enzyme gets inhibited by

curcumin, it slows down the carbohydrate metabolism into simple sugars. Hence, the blood glucose levels do not rise. Ponnusamy and colleagues have reported this mechanism by analyzing bisdemethoxycurcumin kinetic effects. Bisdemethoxycurcumin is one of three main components of turmeric. Bisdemethoxycurcumin acts a potent small molecule inhibitor for human pancreatic α -amylase. This analysis was done in a series of bioassay-guided purification steps. At every step, column fraction was assayed for α -amylase inhibition and active fraction were taken further. At the end, three curcuminoids were formed. One of the curcuminoids was dimethoxycurcumin and other two were bisdemethoxycurcumin (Ponnusamy 2012) and were analyzed for human pancreatic amylase, where inhibition was observed in the presence of bisdemethoxycurcumin (Ponnusamy S, Zinjarde S, Bhargava S, Rajamohanan PR, Ravikumar A, 2012).

Turmeric is shown to increase the pancreas secretion of insulin. Fourteen healthy human beings were recruited for this cross over study in Sweden. (Wickenberg J et al. 2010). Subjects who have thyroid disorders and diabetes mellitus were excluded from the study. Each subject's fasting glucose was measured after a 12 hour fast. Oral glucose tolerance test was used to measure the postprandial plasma glucose response and postprandial insulin response. Subjects were given a total of fifteen 400 mg of turmeric with 170 mg of lactose supplements or just 560 mg of lactose supplements as a treatment and placebo plan for a week long study. Insulin concentrations were measured at 0 min and at 1 hour. This showed that total of 6 grams of turmeric increased the insulin response in the blood stream. The highest peak of insulin was noted at 30th minute.

Compared to the placebo group, turmeric group maintained the increase of insulin in the blood for the entire 120 minutes.

Turmeric and Pharmaceuticals

Curcumin has been studied prevalently in the pharmaceutical world. Drug mechanism of certain drugs used for anti-depressant, anti-coagulant has shown very similar effect with curcumin ingestion. In a study conducted by Bhutani and colleagues (2009) anti-depressant like effect of curcumins with combination of piperine were observed. When 20 and 40 mg of curcumin per kg was given to female Wistar rats for 21 days along with 2.5 mg per kg of piperine, it was observed that there was an increase in monoamine oxidase (MAO) enzyme activity. In addition, it increased serotonin and dopamine concentrations in the body. This suggests that curcumin exerted antidepressant-like effect in chronic unpredictable stress induced depressed rat models ((Bhutani MK, Bishnoi M, Kulkarni SK., 2009). *In vivo* anticoagulant activities of curcumin and its constituents have been studied. Study data suggests that curcumin and increased the Partial Thromboplastin Time (PTT) significantly. It also showed the inhibition of thrombin activities. Hence, this shows that curcumin has an anticoagulant activity (Kim DC, et al. 2012). In another study when curcumin containing 5 and 10% ethanolic extract ointment was given to wounded rats it was observed that there was a significant difference with remarkable healing process against the wounds. This was compared against retardation of wound healing process by 150 mg per kg of aspirin. This is understood due to increase in cellular proliferation and collagen synthesis at the wound

when curcumin ointment is applied. This shows that curcumin has capacity to enhance wound to heal (Pawar RS, Toppo FA, Mandloi AS, Shaikh S., 2015).

Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is a disease that causes sugar levels to go high in blood stream due to body's inability to absorb the sugar and use the insulin, a hormone produced by pancreas. Initially, pancreas tries to compensate the increase in sugar levels in body by producing more insulin but gradually it gets detreated and sugar levels increases. Over time, these high amounts of sugar in the blood produce an adverse effect on the body by slowing the circulatory systems and damaging different organs of the body like heart, kidney, nerves, eyes etc. According to the International Diabetes Federation, by the year 2035 there will be a high rise in the diabetes cases. They have predicted that the number will rise from 387 million to 592 million, a 53% increase. Annually, 4.9 million deaths have been recorded due to diabetes.

Treatment in Type 2 Diabetes

Absence of insulin due to defect in the pancreatic cells or increase in insulin values due to body's inability to respond to it in the presence of sugar in the blood, become insulin resistance over time. This occurrence could be due to uncontrollable function of their liver making insulin. In some conditions, if the insulin cells are damaged they send out wrong information about blood sugar and blood sugar level rises. If diabetes is not diagnosed over time, long term effects like damage of kidneys, blood

vessels, heart, eyes, nerve damage etc. occurs. Hence, it is essential to monitor and manage diabetes to avoid further complications.

Over the time, science has been a boon in treating type 2 diabetes with new medicinal innovations and discoveries. There are now several medications and injections available to control blood glucose and insulin levels in the body. There are more than 20 types of insulin sold in the United States, where each insulin follows a different mechanism that it works in the body. Rapid-acting, short-acting and intermediate-acting and long-acting are the four main types of insulin. These insulins start working in the body from as little as 5 minutes and it can be effective in the body for 12 to 18 hours and some even 24 hours. NovLog[®], Levemir[®], Lantus[®] etc. are the common insulin injections available for diabetic patients. There are six classes of oral medicines and 12 individual drugs available in the United States to control type 2 diabetic patients blood sugar levels. Metformin[®] is the most common drug that is used for more than a decade. It is from a class called the sulfonylureas that is used in the management of type 2 DM. Metformin[®] works by suppressing endogenous glucose production and increasing muscle and fat cell sensitivity for the action of insulin in liver. It reduces insulin resistance and improves glycemic control (Papanas N., Mikhailidis DP., 2009). In addition, metformin has also shown to work favorably on blood pressure, lipids, hemostasis along with improving cardiovascular disease in type 2 diabetes patients (Papanas N., Mikhailidis DP., 2009, Despres JP., 2003). This medication has been recommended by American Diabetes Association (ADA) due to its less side effects like weight gain, hypoglycemic attacks and also blood glucose lowering effect (Hiroyuki Ito et al., 2010). Initially, it was

used to treat obese type II patients (Bell PM, 1997). However, over the course of time, this class of medication works on both, obese and non-obese patients. In a study done on 213 patients with type 2 diabetes it was observed that with the long-term treatment of metformin therapy in non-obese patients gives a beneficial effect. When the effect was compared it was similar to obese patients. HbA1c levels were noted equal between the non-obese and obese groups. In 12 months, HbA1c was reduced by 1.2% in non-obese and by 1.1% in obese patients. Hence, even though the dosage is not similar in two groups, the effect of the drug on the individuals were noted similar when comparing their HbA1c levels (Hiroyuki et al., 2010). Donnelly et al. has reported that metformin is more effective in type 2 diabetic patients with a lower BMI, however with a small clinical impact as the reduction of HbA1c in non-obese patients of 1.46%, was similar to that of obese patients, 1.34%. Hence, this shows that glycemic response to metformin in non-obese and obese patients is similar (Donnelly LA., 2005).

Unlike other pharmacological therapies for type 2 diabetes mellitus (insulin and sulfonylureas), long term consumption of metformin does not lead to weight gain in type 2 diabetic patients. This was observed and analyzed by The diabetes prevention program research group in a 7-8 year follow up in a 2,155 patients taking metformin in a randomized double-blind clinical trial versus placebo. This study took place from 27 various clinics across states. Patients took 850 mg per day of metformin or placebo and then was increased to twice a day. Weight was measured twice every year along with waist circumference, and hematocrit and hemoglobin measured annually. Over the course of time, it was observed that patients who took metformin, their hemoglobin and

hematocrit slightly decreased. Above all it was noted that patients who took metformin had reduced body weight and waist circumference compared to placebo. Thus this shows that over the course of time, metformin helps in weight loss once it is adhere by the patient (Diabetes Care, 2012).

Another class of oral glucose-lowering drug used in the treatment as well prevention of type 2 diabetes mellitus is α -glucosidase inhibitors (AGIs). This class of drug works by altering the intestinal absorption of carbohydrates, enzymes found in the brush border of gut epithelium, into simple sugars to monosaccharides. This is easily absorbed in the intestine and it decreases the bioavailability of carbohydrates in the body (DiNicolantonio JJ, 2015). This effect of AGIs helps in reducing postprandial hyperglycemia (Deros G, Maffioli P.,2012). The three common AGIs used in clinical practice are Arcobose[®], Voglibose[®], and Miglitol[®]. It has been studied that AGIs, particularly Arcobose may be safe and effective for the treatment of prediabetes and diabetes (Deros G, Maffioli P., 2012).

In order to observe AGIs impaired glucose tolerance, Kawamori et al. conducted a study to understand Voglibose[®] in the prevention of developing type 2 diabetes. It was noted from this study that patients who were on Voglibose[®], had significantly lower risk for progression of type 2 diabetes than placebo along with showing normoglycemia in the treatment group. In the study STOP -noninsulin-dependent diabetes mellitys (STOP – NIDDM) study it showed that Arcobose[®] reduced the risk of developing diabetes by 49% (Chiasson et al.2002). Hence, AGIs can help in decreasing the progression of type 2 diabetes along with reducing HbA1c, fasting blood glucose.

Herbs Used in Type 2 Diabetes Mellitus

Prevention of hyperglycemia due to insulin deficiency or insulin resistance has been the motto from ages. Besides allopathic medications, natural alternatives have always been in search to prevent and cure type 2 diabetes. Discovering new antidiabetic agents and finding active ingredients and compounds from herbal plants have been studied since ancient times like in Ayurveda and Unani, ancient Indian system of medicine. Herbs prepared by boiling, extracting juices, fermenting juices and powders have been used in the form of treatment for type 2 diabetes. It is known that a single herb exerts has several different actions on different diseases (Sexena, 2004). Besides, it can also have a synergistic and adversary effects in combination with other herbs (Pendse and Iyengar, 1961, Sexena, 2004). There are few herbs that have been proved scientifically to show positive outcome in the treatment of diabetes. Bitter melon, Fenugreek, Tinospora, and pomegranate are some of the herbal plants and trees that have shown positive results in treating diabetes.

Bitter melon belongs to the Cucurbitaceae family and is grown in tropical areas in India, Asia and South America (Sexana, 2004). Its juice has been used as a hypoglycemic agent in Indian ancient medicine (Sharma, 1996, Sexana, 2004). It was observed *in vitro* that bitter melon juice has an inhibitory effect on glucose absorption in the intestine (Meir and Yaniv, 1985, Sexana, 2004). Higashino et al. observed that it also enhances insulin release from beta cells (Higashino et al., 1992). In addition, Welihinda et al. also noted that bitter melon increases glucose uptake by tissue and has an extra pancreatic effects by increasing beta cells that helps in regulating blood glucose levels

(Welihinda et al., 1998). It has been studied that phytochemicals of bitter melon have insulin mimetic activity. Murthy et al. (2002) studied the phytochemical compounds and found that when 400, 300, and 100 mg/kg per day doses were ingested in mildly diabetic rabbits, hypoglycemic effect was observed in them. This effect is speculated due to their galactose-binding lectin, insulin-like protein present in them (Ng et al., 1986, Saxena, 2004). In the study conducted on eight-insulin dependent participants it was observed that when 4 g of bitter melon powder was ingested for 21 days, postprandial blood glucose level was decreased significantly (Habib A., 2003).

Fenugreek is another herb plant, in the Leguminosae family that has been used in type 2 diabetic treatment. It is a native to western Asia and southeastern Europe and is cultivated in Mediterranean region, China, and northern India (Saxena A., 2004). Studies on rats have shown diminution in fasting blood glucose by approximately 80% (Saxena A., 2004). Khosla and colleagues studied the effect of 2 and 8g/kg of fenugreek dose in diabetic rats. It was observed that the blood glucose levels were decreased significantly. Interestingly, in this study normal non-diabetic rats blood glucose was also decreased at a given dose (Khosla P. Gupta DD, Nagpal RK). This shows that ingestion of fenugreek may be beneficial in controlling blood glucose levels in normal as well diabetic individuals. In a double blind placebo controlled study conducted with 25 mild type 2 diabetic patients showed an improved glycemic control and decrease insulin resistance. In this study 1 g/day extract of fenugreek seed (hydroalcoholic extract) were given to 12 patients and were compared with 13 other patients who received placebo capsules. When results were measured after 2 months, it was observed that consumption of fenugreek for

2 months have decreased the insulin significantly (Gupta A., Gupta R., Lal B.,2001).

Hence, this shows that when fenugreek is taken in type 2 diabetes as an adjunct therapy it can help in improving glycemic control and decreasing insulin resistance.

Tinospora is a member of the Menispermaceae family and is grown in India, Myanmar and Sri Lanka (Saxena, 2004). Traditionally its juice has been used in diabetic patients. In the study done on albino rats, ingestion of 2.5g/kg and 5.0 g/kg dose of root extract, showed decrease in fasting blood glucose. (Stanely et al.,2000) The reduction was noted of approximately 130mg% after the supplementation. It has been noted from different studies that tinospora root extract decreases the levels of ceruloplasmin, alpha-tocopherol in diabetic rats along with increasing Vitamin-C concentrations which acts as an inhibitors of free-radical-mediated lipid peroxidation (Prince et al.,1999. Meistor and Anderson, 1983, Saxena, 2004).

Pomegranate is a fruit filled with rich-red colored juicy seeds. It is a small tree that is from the Lythraceae family originated in Asia and is widespread in Mediterranean regions, South Africa and China (Saxena, 2004). Flower, seeds, rind are this plant's part that are known to have an antidiabetic effects. In the study conducted on diabetic rats, it was observed that when seed extract doses of 300 mg/kg and 600 mg/kg were given, blood glucose levels were reduced by 47% and 52% respectively within 12 hours (Das et al.,2001). When flower extract was administered to a normal, glucose-fed and streptozotocin-induced diabetic rats, it was found that the extract of 400 mg/kg decreased blood glucose level effectively. However, the mechanism is yet unclear with a speculation that it may increase peripheral glucose utilization (Jafri et al.,2000, Saxena,

2004). Sharma et al., (1983) also suggest the mechanism that may inhibit proximal tubular reabsorption for glucose in kidney. (Sharma et al.,1983, Saxena, 2004).

Herbs and plants have shown to have many health beneficial properties especially the one discussed above in controlling blood glucose levels in the body. This herbs exhibit hypoglycemic, hypolipidemic and antioxidant properties in animals and humans. However, these observations are on few studies and mostly on animals. Thus, more detailed study should be conducted on these herbs to evaluate the exact mechanisms that each one works on the body as there may be several active ingredients present in them.

Cinnamon is a bark, that is obtained from several trees belonging to the family of Lauraceae (Iqbal, Mohammad, 1993). It is used as flavoring spice. Studies have shown that consumption of cinnamon spice helps in controlling fasting blood glucose in type 2 diabetic patients. Study conducted by Khan et al. (2003) showed that when 1, 3 or 6 g per day of cinnamon was added in 60 type 2 diabetic patients diet for 40 days, their fasting blood glucose decreased. The reduction of fasting blood glucose was the most statistically significant for the dose of 6 g per day of cinnamon ingestion. In a review article of clinical trials Allen et al. concluded that cinnamon is associated with a statistically significant decrease in fasting blood sugar along with total cholesterol, LDL and triglyceride levels. However, it was noted that cinnamon consumption does effect the HbA1c in diabetic patients (Allen et al., 2013). As for now, the active ingredient lowering the blood glucose has not been identified. Hence, the type of cinnamon and in which form i.e. powder, capsule, or extract along with the dosage of cinnamon is not yet confirmed.

Minerals and Management of Type 2 Diabetes

Magnesium is one of the essential mineral and is a cofactor for hundreds of enzymes as it is involved in several physiologic pathways, including energy production, protein synthesis, cell signaling, ion transport, glucose metabolism and many more (Harper et al., 2010). Magnesium deficiency has been associated with developing type 2 diabetes mellitus due to systemic inflammation and its role in glucose homeostasis and insulin action (Saris NE., 2000, Barbagallo M, 2003, Larsson SC.,2007). Magnesium is essential for the activation of phosphate-dependent enzymes in the glycolytic pathway and Krebs cycle in the pancreatic β -cells. It is also essential for the transcription of nuclear protein factors for the release of insulin. It is understood that insulin function is magnesium dependent as it activates the β -subunit of tyrosine kinase domain of the insulin receptor and stimulates proteins and substrates in the insulin-signaling cascade (Harpers, 2010, Sales et al., 2010). From animal studies it has been observed that insulin secretion and action is impaired if there is magnesium deficiency in the body (Suarez A et al., 1995, Balon TW et al.,1995). By supplementing magnesium, the incidence of type 2 diabetes decreased in animal studies. However, this has not completely braced in the human studies. It has not been fully supported to show that magnesium supplementation benefits the effect on glucose metabolism, insulin action and/or insulin sensitivity. However, cohort studies have shown an inverse association between 100 mg per day of magnesium supplement and developing risk of type 2 diabetes by 15% (Larsson SC.,2007). In a study, conducted by Sales CH et al. (2011) on 51 type 2 diabetic patients, it was observed that magnesium status was altered in them (Sales CH, 2010). In a meta-

analysis study of 13 observational studies it was found that higher magnesium intake was associated with a lower risk of diabetes (Dong JY et al., 2011). As it has been understood that pancreatic β -cells regulate insulin secretion and glucose tolerance and if there is magnesium deficiency then the insulin sensitivity can decrease. In a randomized, double-blinded, placebo-controlled study, 97 healthy, non-diabetic participants with hypomagnesemia were studied. When 638 mg of magnesium solution were administered for three months, their fasting blood glucose and insulin levels decreased. This shows that pancreatic β -cell function was improved with the ingestion of magnesium (Romero F., et al, 2011). In another study by Mooren FC and colleagues, it was observed that when 365 mg per day of magnesium supplement from magnesium aspartate hydrochloride was given for six months in 47 overweight individuals their insulin resistance was decreased (Mooren FC, et al., 2011). Thus, this suggests that magnesium supplementation may have some effect that can help in preventing type 2 diabetes by decreasing insulin secretion. In addition, it also shows that when type 2 diabetic patients who are deficient in magnesium, supplementing them the dosage of magnesium 100 mg per day capsules along with regular diabetic medications may help in controlling blood glucose levels.

Chromium is an important trace element in the body and has been proposed to be the cofactor for enhancing the effects of insulin on target tissues. It is an essential nutrient that takes place in lipid and carbohydrate metabolism (Anderson RA., 1997).

Chromodulin has been postulated to have an enhancing cascade effect in the process of insulin binding to extracellular α -subunit of the insulin receptors. In type 2 diabetes

patients, there is a high loss of urinary chromium when compared with healthy individuals (Morris BW et al.,1999). Anderson RA and colleagues showed that chromium supplementation might be beneficial in the treatment of type 2 diabetes as it reduced insulin concentration compared to placebo in the study. In this randomized study, one hundred and eighty type 2 diabetic patients were given chromium supplements in the form of chromium picolinate at 100 mcg per day or 500 mcg twice per day for four months. It was observed that HbA1c levels improved significantly in the group taking 100 mcg chromium per day. Fasting blood glucose levels were also decreased after four months of the supplementation. There was a significant decrease in fasting and 2-hour insulin values in both the groups receiving 100 mcg per or 500 mcg per day of chromium supplementation. In a meta-analysis of placebo-controlled studies it was concluded that by 250 mcg per day of chromium supplements for at least three months, fasting glucose concentrations can be reduced. However, in this analysis of seven studies, there was no difference in the HbA1c levels in the diabetic patients (Abdollahi M et al. 2013). Hence, even though chromium is an essential element in the carbohydrate metabolism, studies have not concretely suggested that chromium supplementation can help as an adjuvant therapy or as a preventative therapy in type 2 diabetes. In addition, chromium has a low bioavailability than of dietary chromium, hence taking it in a large quantity may not be safe for a long-term use (Food and Nutrition Board, 2001). Thus, more studies with different doses of chromium are required to observe the beneficial effects of supplementation in the treatment and prevention of type 2 diabetes.

Turmeric as an Adjuvant Therapy in Type 2 Diabetes

Studies have now concluded that turmeric as well as curcuminoids can help with type 2 diabetic mellitus by either lowering blood glucose or increasing insulin sensitivity. Selvi and colleagues (2015) used turmeric along with Metformin® in type 2 diabetic patients to see the beneficial effect of medication as well as turmeric. For this study, sixty type 2 diabetic subjects who were on metformin therapy were selected. The study was conducted as a randomized trial in which there were two groups of thirty subjects each. As a treatment plan, one group just got 500 mg of metformin therapy twice a day, whereas another group received 500 mg metformin therapy twice a day as well as 2 grams of turmeric supplementation for 4 weeks. The turmeric capsules were ingested after two of metformin administration. In order to measure the blood sample overnight fasting blood was recorded at the beginning of the study, and after four weeks of study. From the lab data's it was observed that subjects who took turmeric supplements along with regular metformin therapy, fasting blood glucose was significantly lowered by 15% when compared with only metformin therapy which was only of 6%. In this study, no statistically significant difference was measured in the post prandial glucose. However, HbA1C was observed by 5% in the people who took turmeric supplementation. In addition, when oxidative stress was measured turmeric supplementation showed an increase in glutathione levels more than just metformin therapy. This showed that turmeric has an antioxidant property when total antioxidant capacity was measured and compared with only metformin patients. In addition to this study results it also showed

decrease in lipid levels especially LDL cholesterol levels in patients who took the turmeric supplementation. (Selvi 2015).

Conclusion:

The above researches has provided the scientific basics for turmeric and curcumin in the role of prevention and treatment for diabetes as well as other diseases. There are several mechanisms that have been proposed by which curcumin may exert its effect on blood glucose and insulin regulation in rats. However, more research is required to find the exact mechanism of action. It has been observed that curcumin can help in insulin resistance, hyperglycemia, hyperlipidemia and other related disease factors in humans. From the analyzed mechanism by Ponnusamy and colleagues it shows a good promising outcome for the treatment of type 2 diabetes, which regulates starch digestion in the body. Hence, it is important to differentiate the possible different process that turmeric has in order to show the effect on glucose regulation.

CHAPTER 3

METHODOLOGY

Participants

Participants were recruited from Arizona State University, downtown campus and nearby community. Verbal announcements, electronic messages, and posted flyers were used to enroll participants for the study. In order to confirm the number of participants that should be used for the study, power analysis calculations were completed (Appendix A). Eight healthy subjects: eight female participated in this study. All subjects met enrollment criteria for age (21-75 years (mean: 25 ± 11 y) and body mass index (BMI) ≥ 25 kg/m² (mean 24.7 ± 2.5 kg/m²). This study included healthy, maintain stable weight for past three months (± 3 kg; mean: 68.7 ± 8.9 kg), non-vegan, non-vegetarian, and nonsmoking men and women. In addition, patients who are not diagnosed with any irritable bowel, celiac disease, gall bladder disease, history of kidney stones and have not done any medical procedures related to gastrointestinal tract were included. It was made sure that women participants are not pregnant or have not become recently pregnant and/or are lactating. All participants showed willingness to follow the screening process that includes height, weight, waist circumference, and BMI will be measurement. They followed the 2-hour study protocol that includes eating mashed potato test meals, drinking 8 oz. juice (4 oz. orange and 4 oz. beet juice) during their first initial visit of screening. All participants were screened for their willingness to ingest test meals, provide blood samples and participate in anthropometric data collection. from finger pricks and plasms insulin were collected to measure blood glucose and insulin levels.

Written informed consent (Appendix B) and a medical questionnaire were completed by each subject prior to the commencement of the study. The Arizona State University Institutional Review Board, Human Subject Committee approved this research prior to initiation of recruitment.

Study Design

The study followed a randomized, double-blind, crossover design. It comprised of two distinct parts. The study was conducted for a 9-week period.

Part I: conducted during the weeks of 1-3 during which participants consumed a bagel the night before coming to the testing center. They reported to the site in a 12 hour fasting state and received a test meal (mashed potato + 8 oz. of orange and beet juice) with 500 mg turmeric or placebo of corn meal. (See Tables 1 for details regarding test meals composition, Appendix D test meal ingredients and Appendix E for test meal recipe). 500 mg of treatment or placebo capsules were given to the participants to take home and were instructed to take one supplement once a day with meal.

Part II: this part was during the weeks of 4-6weeks where participants were under the washout phase. No medication or placebo treatment were given to participants to generate participants' baseline data.

Part III: conducted during the weeks of 7-9, by utilizing a randomized cross-over design. This time the participants who received the turmeric treatment plan received the placebo and who received the placebo got treatment plan. Participants were instructed to

consume a bagel the night before coming to the testing center. They reported to the site in a 12 hour fasting state and received a test meal (mashed potato) with 8 oz. of orange and beet juice mixed with 500 mg turmeric or with placebo of plain orange and beet juice. (Table 3 provides the detail of participants' randomization using a coin; T = tail treatment (turmeric), H = head → placebo; all participants were assigned a number from 1-15).

Table 1. Participants treatment randomization.

Randomization								
Participant #	2	4	6	8	9	10	11	13
Phase I (week 1-3)	T	T	H	H	H	T	T	H
Phase III (week 10-12)	H	H	T	T	T	H	H	T

T = tail → treatment

H = head → placebo

N = 8

Table 2. Test meals composition for mashed potato and orange + beet juice

	Weight	Calories	Carbohydrate	Fat	Protein
Mashed Potato: Russet Potatoes ¹	100 grams	79 kcal	73 kcal	0.7 kcal	5.9 kcal
Butter	50	214 kcal	0.07 kcal	213.2 kcal	1.02 kcal
Cream	50	173 kcal	167 kcal	9.23 kcal	5.58 kcal
Salt	¼ teaspoon	0	0	0	0
Pepper	¼ teaspoon	0	0	0	0
Juice					
Orange juice	4 oz.	55 kcal	52 kcal	117 kcal	52 kcal
Beet juice	4 oz.	40 kcal	40 kcal	90 kcal	40 kcal
TOTAL	----	561 kcal	332.07 kcal	430.13 kcal	104.5 kcal

Data collected using skipthepie.org

The Food Processor, ESHA Research Version 12.11

On each day prior to every data collection, participants were instructed to consume dinner that can include chicken, fish, turkey or eggs with bread and/or rice along with the provided bagel. Participants were asked to brush and floss their teeth

thoroughly the night before the test and the morning of the test. In addition, they were asked to consume dinner no later than 12 hours before the testing. Participants were refrained from any heavy exercise prior to the testing day. No other food with the exception of water was consumed after this dinner meal and participants were in an overnight fasting state until they arrived at the research center ABC building, ASU Downtown Phoenix Campus the next morning.

Upon arrival, a fasting blood sample were collected to determine both the fasting serum glucose and insulin values for each participant. During the weeks 1-3, participants consumed the test meal of mashed potatoes and juice along with either turmeric or with placebo.

Once the participant took the first bite of test meal, time was marked as 0 and was considered as a starting point for the 2-hour test. Participants did not consume anything during this time besides water. They did not move, but were allowed to read or work on their computer. After thirty minutes of testing meal, a finger prick was performed using ACCU-CHEK *Aviva Plus* glucometer 2 (LifeScan Inc., Milipitas, CA). The same glucometer was used throughout the study. Thereafter, blood draws were performed and drawn at 30, 60, 90, and 120 minutes after test meal consumption. At 0 min and 30 min plasma blood samples were collected to measure the insulin. This data collection procedure was repeated for each participant for total of six weeks. The test meal, juice and turmeric were administered by the participants themselves.

After the completion of the test meal and data blood collection, participants then left the test site and were free to resume their normal activities along with their dietary behaviors. Subjects were given turmeric or placebo supplements to take for the next three weeks. They were instructed to take the supplement with their first meal.

Food Analysis

Food items to make mashed potato and orange juice and beet juice were purchased from Safeway grocery store. In addition, turmeric was purchased in a powder form from Whole Food grocery store. The dose of the turmeric that was used in this study is based on the amounts previously used in a similar study design.

Anthropometric Measurements

Body weight, height and weight circumference measurements were obtained for each subject at each of the screening visits. Body weight and height were measured with a calibrated scale (Tanita Tbf 310 Professional body weight Scale), with the subject wearing light clothes and no shoes. BMI was calculated as kg/m^2 . Waist circumference was measured at the level of the umbilicus, by using a spring adjusted Gulick II measuring tape (Country Tech. Inc., Gays Mills, WI) and is reported in inches.

Blood Analyses

Blood draws for serum glucose were obtained from each participant at: fasting, and then at 30, 60, 90, and 120 minutes' time interval after the test meal with juice and turmeric or placebo. In addition, one capillary glucose measurement was also obtained at zero and 180th minute. A calibrated ACCU-CHEK *Aviva Plus* glucometer (LifeScan Inc., Milipitas, CA) was used to measure capillary glucose values. A trained phlebotomist and RN performed all the blood draws throughout the study.

Statistical Analyses

Statistical analyses of the results were computed using the Statistical Package of Social Sciences (SPSS) version 23.0 by SPSS Inc. (Chicago, IL). Statistically significant repeated measure ANOVA was used to find out the significance between the two groups. The level of significance will be set up to $p \leq 0.05$. Normality for area under the curve (iAUC), insulin and HOMA-IR was measured. Data was normally distributed based on Shapiro-Wilk test ($p > 0.05$) for all of the groups. Means and standard error (SE) are reported for each variable analyze.

CHAPTER 4

RESULTS

Descriptive Characteristics

Twenty-nine participants responded to the online survey for this study that examined the effect of turmeric on postprandial glucose and insulinemia in normal healthy participants. Among them, nineteen qualified for the initial online screening. Eleven participants (11 females) were consented for the study, where one did not qualify for the study and one had transportation conflict. Thus, nine participants (9 females) took part in this study. Randomization of the participants was done by flipping the coin, T= treatment (turmeric) and H= placebo by the volunteers helping with the study. Since, this was a cross-over study design, five participants received treatment and four participants received placebo for their visit 2. After the washout period of 5 weeks, participants who received treatment were given placebo and who received placebo were given treatment. Data were collected for all 5 visits from all nine participants with the exception of visit 4 and 5, during which participant #3 was not able to participate due to her gluten sensitivity issue. Therefore, due to the absence of participant #3 data, n=8. Table 1 shows the baseline characteristics of the study participants.

Table 3. Descriptive characteristics of participants measuring the effect of turmeric¹

	N	Minimum	Maximum	Mean	Std. Deviation
Age	8	18.0	45.0	24.8	10.6
Weight, kg	8	51.5	81.8	68.7	8.9
Height, cm	8	155.0	179.0	166.8	8.0
BMI, kg/m²	8	21.4	27.8	24.7	2.5
Fat, per	7	15.3	38.4	30.8	8.0
Average fasting glucose (um/mL)	8	9.26	20.5	15.4	4.3
Average fasting insulin	8	9.3	20.5	15.4	4.3

¹Average mean for cross over

Postprandial Blood Glucose Response

Blood glucose was collected during the meal testing via finger prick. Figure 1 shows the blood glucose response for 0-, 30-, 60-, 90-, and 120 minutes after treatment administration. Using the repeated measure ANOVA analysis, no statistically significant difference in postprandial glycemia between groups was noted ($p=0.835$; effect size 0.278) (Table 2 and Figure 1). Incremental area-under-the-curve (iAUC) for fasting glucose were calculated using the trapezoidal rule and there were no statistical significant differences for the iAUC glucose response between the turmeric treatment and placebo group ($p=0.564$; effect size 0.050). However, there was a 4.4% reduction in fasting glucose after ingesting turmeric treatment for 3 weeks. The effect size was large, 0.165.

Hence, there may be some physiologically relevance of this change; however, this difference was not statistically significant ($p=0.278$). Figure 2 shows the blood glucose comparison between week 0 and week 3 among the treatment and placebo group.

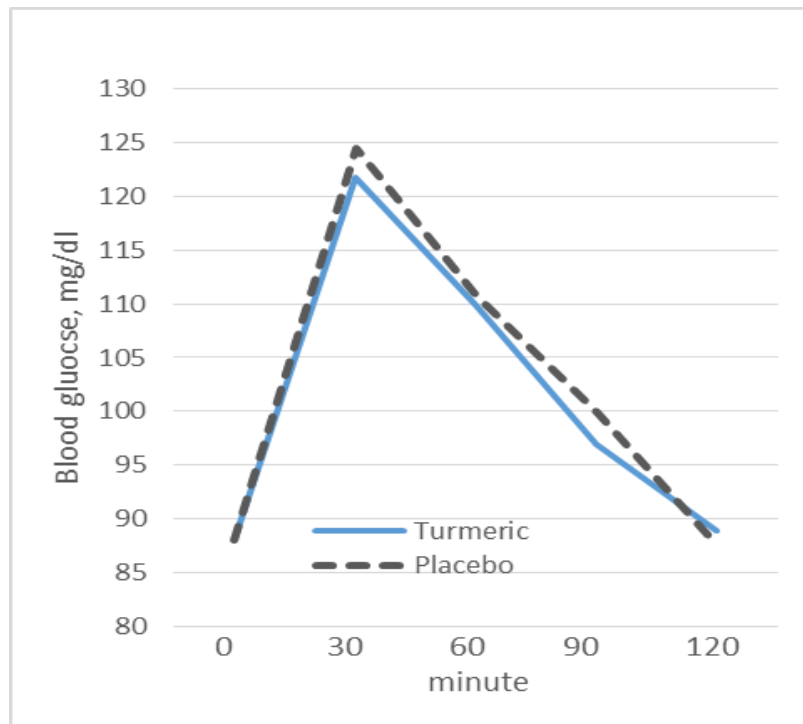


Figure 1 - Postprandial blood glucose concentrations during 120 minutes of the blood glucose test, after test meal consumption under treatment and placebo conditions. Values are means \pm SD.

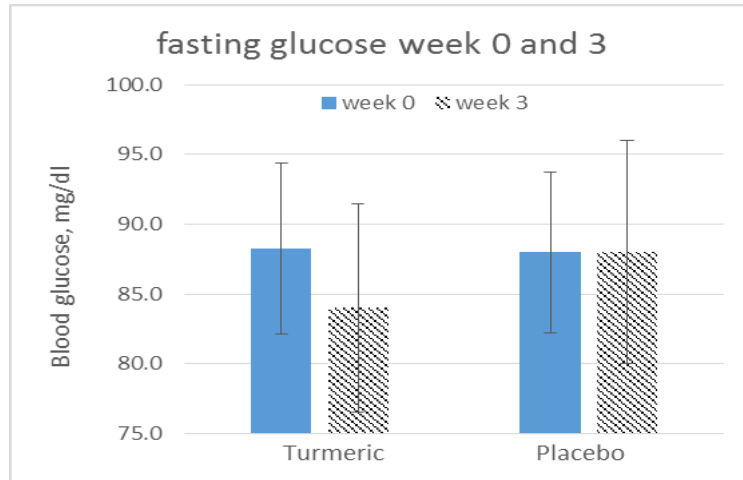


Figure 2 - Fasting blood glucose concentration comparison between treatment and placebo, during week 0 before treatment and week 3 after treatment. Values are means \pm SD

Table 4: Fingersticks data for postprandial glucose response for 120 minutes and 3 week fasting glucose following test meal consumption in healthy adult participants^{1,2,3}.

<i>Postprandial fingersticks (mg/dl)</i>				
	Placebo	Treatment	<i>p</i> -value	Effect size
0 minutes	88.00 \pm 7.5	88.25 \pm 6.1		
30 minutes	124.50 \pm 19.4	121.75 \pm 13.8		
60 minutes	110.75 \pm 19.3	109.88 \pm 13.1		
90 minutes	99.88 \pm 9.2	97.00 \pm 10.8		
120 minutes	87.50 \pm 5.5	88.88 \pm 8.9	0.835	0.278
iAUC	70.86 \pm 39.5	64.19 \pm 20.2	0.564	0.050
3-week glucose	87.88 \pm 7.9	84.00 \pm 5.8	0.278	0.165

¹ Data are mean \pm SD

² P values represent repeated measures ANOVA

³ N= 8

Plasma Insulin Response

Plasma insulin was evaluated at baseline (fasting) and 30 min post-test meal consumption. No significant differences were observed for insulin response between groups following consumption of test meal ($p=0.524$; effect size = 0.060) and placebo (Figure 3). Repeated measures ANOVA was conducted to compare the fasting insulin concentrations before and after 3 week of treatment administration by group. It was noted that the data were not statistically significant ($p=0.814$; effect size = 0.008) (Figure 4).

Insulin sensitivity were measured by calculating Homeostatic Model Assessment of Insulin Resistance (HOMA -IR). Baseline and 3-week HOMA-IR scores were compared. It was noted that there is no significant difference between the two data sets and are not statistically significant ($p=0.649$) with a small effect size (0.031) (Table 3).

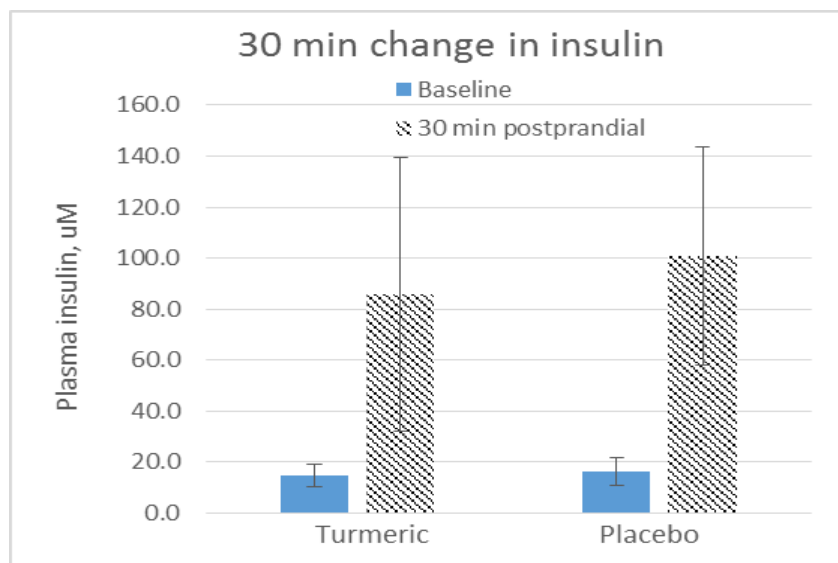


Figure 3 - Plasma insulin comparison between fasting and 30 min postprandial insulin levels after test-meal consumption for treatment and placebo groups. Values are means \pm SD

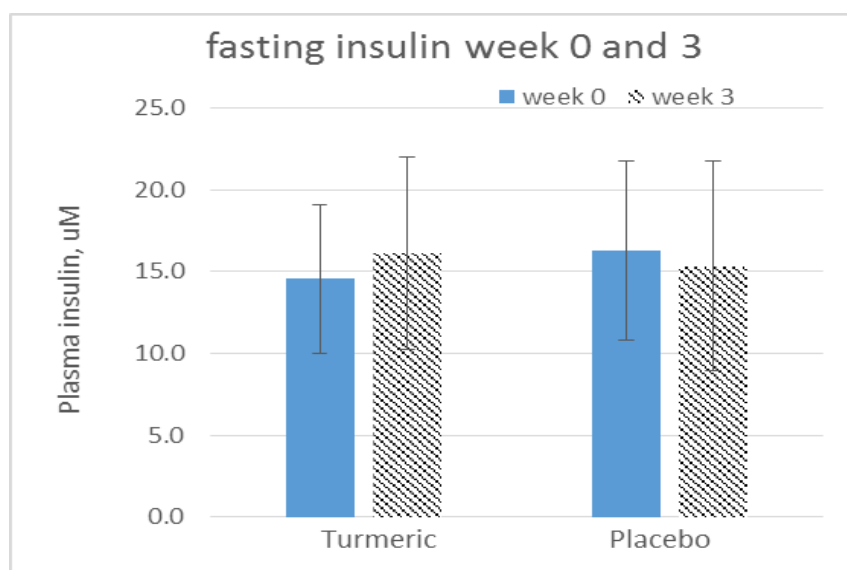


Figure 4 - Fasting plasma insulin concentration comparison between treatment and placebo, during week 0 before treatment and week 3 after treatment. Values are means \pm SD

Table 5: Plasma insulin data for 0 min and 3 weeks in healthy adult participants^{1,2}.

<i>Plasma insulin (U_m/ml)</i>				
	Placebo	Treatment	<i>p</i> -value	Effect size
0 minutes	16.3±5.5	14.6±4.6		
30 minutes	100.9±42.8	85.8±53.9	0.524	0.060
3-week insulin	15.3±6.4	16.1±5.9	0.814	0.008

¹Data are mean ± SD

²P values represent repeated measures ANOVA

Table 6: HOMA-IR scores for baseline (0 min) and 3 weeks for treatment and placebo groups of healthy adult participants^{1,2,3}.

<i>HOMA-IR Scores</i>				
	Placebo	Treatment	<i>p</i> -value	Effect size
Baseline	3.56±1.35	3.15±0.94		
3-week insulin	3.33±1.40	3.33±1.19	0.649	0.031

¹Data are mean ± SD

²P value represent repeated measures ANOVA vs baseline values

³HOMA-IR = Homeostatic Model Assessment of Insulin Resistance

CHAPTER 5

DISCUSSION

Animal studies have shown that curcumin has a controlling effect on blood glucose (Zang, 2013). Our study looked at the consumption of turmeric, which consists of an active ingredient curcumin, in healthy adults which failed to show a decrease in postprandial glucose and insulin levels. This results in our study could be due to small n , resulting in deficient power to detect significance. It may also be possible that participants did not comply with data protocols (exercise and consumption of control meal prior to data collection, missing the dose of treatment capsules over the 3-week period). Similar to the study done by Wickenburg and colleagues, there were no significant difference noted in blood glucose between placebo and treatment group ($p=0.564$). Karpaga et al. also noted in their study that there is no statistically significant difference in post prandial glucose levels, when compared within and between the turmeric and placebo groups in type 2 DM patients. No significant changes were noted in postprandial insulin response in our study between the treatment and placebo group ($p=0.524$, effect size 0.060). This may reflect the controlling levels of glucose and insulin respectively. Conversely, in the study done by Wickenburg and colleagues (Wickenburg et al. 2010) serum insulin levels increased after the ingestion of turmeric. Hence, this change in our study shows that turmeric may have an effect on insulin secretion. Our study failed to show a significant difference in insulin levels as it may be possible that the dosage of treatment was lower than the previous study i.e. 0.05 grams versus 6.0 grams respectively.

Comparison of fasting blood glucose in three-week data between turmeric and placebo was also not statistically significant ($p=0.278$) but showed to have a large effect size (0.165) in our study. This suggests that there may be some physiological relevance with the treatment group. Unlike participants with type 2 DM taking Metformin[®], administering turmeric powder in supplement form for 4 weeks showed significant reduction in fasting plasma blood glucose when compared with placebo (Karpaga et al. 2015). The reduction was noted of 15% for turmeric supplementation group and 6% for non-turmeric (placebo) supplementation group. This shows that turmeric consumption has a long term effect and not acute effect on blood glucose levels. No significant change was noted when comparing baseline insulin levels with 3-week insulin levels in turmeric administered participants. Karpaga et al. also noted in their study that turmeric had no significant difference in the fasting plasma insulin. The effect size for 3week insulin was small (0.008) in our study. Hence, this shows that there is no change in insulin levels with long term consumption of turmeric.

In order to asses β -cell function and insulin resistance (IR) from fasting blood glucose and insulin, HOMA-IR for baseline and 3 weeks were compared in our study. This showed no statistical difference between the turmeric and placebo group ($p=0.649$) with a small effect size (effect size = 0.03). Similar results were observed in Karpaga and colleagues study when 2 g of turmeric was administered in the treatment group along with Metformin[®] and were compared with Metformin[®] alone.

Sharma et al. reported in a review article that on average 1.5 g/person/day turmeric is consumed on a daily basis in Asian countries and communities like in India

(Sharma 2005). Hence, when comparing the dosage of our study and other studies ranging from 400 mg to 6000 mg it suggests that in order to see better outcome from turmeric consumption, high amount of turmeric should be administered. In research, curcuminoids, an active ingredient of turmeric contains between 2 to 8% of curcumin (Tayyem 2006, Estbeyoglu 2012, Karpaga 2015). It has shown to have anti-inflammatory, antioxidant, and hypolipidemic activities along with anti-bacterial, anti-viral as well as anti-cancer properties. Curcumin content of turmeric was measured in a study by Tayyem and colleges (2005). Samples of turmeric and curry powder of several brands were compared and it was noted that pure turmeric powder had the highest curcumin concentration, averaging 3.14% by weight (Tayyem, 2006). In turmeric powders, the average curcumin content (% of the dry weight), was 1.51%, ranging from 0.58% to 3.14% showing a 5.4-fold difference (Tayyem, 2006). Hence, when turmeric is ingested as a treatment, its active ingredient curcumin varies and it may not be able to have the same anticipated effect.

Turmeric, a yellow colored spice containing curcuminoids has been studied over the years. Most of the research is done on curcumin, an active ingredient of turmeric containing between 2 to 5% of the spice. Commercial available brand of turmeric capsules contains turmeric as well concentrated form of curcuminoids. Dosage varies from 500 mg to 1000 mg containing various constituent of curcuminoids that should have at least 95% of curcumin to offer therapeutic benefits. If turmeric is taken in the form of spice for treatment, then it should be taken in a sufficient amount to get the active ingredient curcumin. However, it is difficult to increase the turmeric powder

consumption due to its earthy taste and low bioavailability. In our study, 500 mg of turmeric ingestion did not show a significant change in the blood glucose and insulin levels. One of the reasons this could be is due to the variability of curcumin in the turmeric powder. By calculating the curcumin content in 500 mg of turmeric assuming that it contains 3% of curcumin, our turmeric powder supplement had 15 mg of curcumin. Thus, it is essential to know the curcumin content in turmeric powder to more accurately assess the content of the active ingredient in order to enhance the possible beneficiary health effects (Tayyem, 2006). Hence, our turmeric powder was lower in curcumin content when compared with commercial brand curcumin supplements. This shows that if one is recommended to take turmeric as an adjuvant therapy, curcumin supplementation with active ingredients of curcuminoids will be more beneficiary.

Bioavailability of turmeric curcuminoids is very low, especially if ingested orally (Estabeyoglu, 2012). Various animal and human studies have identified the absorption rate of curcumin. It has been noted that small intestine has a very low absorption rate, yet with the highest concentration and liver has a very fast metabolism of curcumin (Washlstrom B, 1978, Shoba G, 1998, Estabeyoglu, 2012). This can explain our study result showing no significant change in postprandial blood glucose levels. In the study done by Washlstrom and colleagues in Dawley rats, it was noted that after the oral administration of curcumin, most of the curcumin is eliminated through the feces, which was unchanged. (Washlstrom B, 1978. Estabeyoglu, 2012). It has also been noted in studies that human plasma shows high doses of curcumin content after 1 to 2 hour of ingestion (Shoba G, 1998, Sharma R.A., 2001, Lao C.D., 2006, Vareed S.K., 2008,

Estabeyoglu, 2012). This suggests that, high dose of curcumin is better for treatment due to its reduced amount of solubility. In Indian cuisines, turmeric is consumed in daily as a cooking spice. It is added in most of the lentils soups, cooked vegetables, rice, and sometimes in various kinds of breads. There are also other spices mixed into the food, but turmeric is considered on the more important spices when during preparation. Turmeric is added as one of the spices to prepare food items. There are other spices mixed into the food along with turmeric. In order to increase turmeric bioavailability in the body, various methods have been studied. Piperine an active component in pepper has been found to increase turmeric bioavailability by two folds (Arcaro 2014). Hence, adding black pepper along with turmeric can help in bioavailability. Oil or some kind of fat is usually present in these cooked food items. Thus, due to the lipophilic property of turmeric, adding fat like coconut oil, corn oil, olive oil etc. in the dish may help in increasing the absorption of the active ingredient curcumin.

Turmeric bioavailability can be enhanced with heat (Kurein, Singh, Matsumoto, Scofield, 2007). Kurein and colleagues study demonstrates that when heat is given there is an increase in the water solubility of turmeric as well curcumin. It was noted that the solubility increased by 12 fold in curcumin and threefold increase in turmeric when it was heated for 10 minutes in a boiling water bath at about 90 degrees Celsius. However, it also showed that following the incubation of heated curcumin samples at 4 degrees Celsius, stability decreased by 47% and 67% after 12 and 72 hours respectively. When turmeric samples were incubated after heating, its stability decreased by 17% and 25% after 12 and 72 hours respectively (Kurein et al). It was also noted that heat did not alter

the molecule structure significantly. In addition, higher peak was observed in the study for one of the components of curcumin, bisdemethoxycurcumin. The peak of curcumin heat treatment stability showed a higher peak in a heated sample than in the non-heated sample. Thus, this shows that heat protects curcumin from breaking down fast. Hence, if we heat the turmeric powder before ingestion, it may help in increasing the solubility in the body.

Our test meal contained Russet mashed potatoes made from cream, milk, butter, salt and pepper. Ponnusamy et al. suggested in their study that one of active ingredient of turmeric bisdemethoxycurcumin (BDMC) acts as a human pancreatic α -amylase (HPA) inhibitor. 10% BDMC was present in the sample and it was noted to hydrolyze the starch, working as a small molecule HPA inhibitor (Ponnusamy et al. 2012). This effect was not observed in our current study statistically, however postprandial blood glucose levels decreased with a small effect size and insulin with a medium effect size. This could support the Ponnusamy et al. *in vitro* study that BDMC may have acted as HPA inhibitor during the test meal time period that consists of Russet mashed potato, high in starch content. In addition, as there was butter and pepper added to the recipe, bioavailability of turmeric might have increased to give a small and medium effect size on the postprandial glucose and insulin response respectively.

Sharma and colleagues reported that more than 2,400 metric tons of turmeric is imported into United States yearly (Sharma RA, 2005, Tayyem, 2006). This shows that it will be beneficial to know the amount of curcumin consumed in form of turmeric as a spice to observe the biological effects.

In conclusion, our study did not support our hypothesis. Turmeric consumption did not significantly reduce post prandial blood glucose levels when turmeric was ingested. This may be due to glucose levels been strictly regulated in glucose tolerant healthy participants. When three-week turmeric consumption results were analyzed it did not show any significant change in blood glucose levels, however; it did appear to attenuate when compared with placebo with a smaller effect size. Our study failed to show any significant change in fasting plasma insulin levels when compared with placebo after three weeks of turmeric supplementation. However, it did show a larger effect size suggesting that long term consumption of turmeric may have some positive effect in insulin levels. Thus, it was observed that turmeric consumption has more of a long term effect than of an acute, short term effect. More studies are needed to measure the long term administration of turmeric and its effect on blood glucose and insulin levels. If this crossover study is repeated, recruiting 25 to 30 participants will give 80 percent probability measuring a treatment difference change of 3.8 units. Future research is essential to examine the turmeric powder supplement benefits over a long period of time in blood glucose and plasma insulin levels in healthy adults. This may help in preventing the occurrence of type 2 diabetes.

Furthermore, studies may want to evaluate the administration of turmeric to subjects with more abnormal parameters such as insulin resistant, impaired glucose tolerant or prediabetes. In addition, turmeric supplementation along with some kind of fat and warm food should be experiment to observe the solubility. More research is

required to determine the mechanism that may be involved in possibly increasing insulin levels with the provision of turmeric.

REFERENCES

1. Abdollahi M, Farshchi A, Nikfar S, Seyedifar M. Effect of chromium on glucose and lipid profiles in patients with type 2 diabetes; a meta-analysis review of randomized trials. *J Pharm Pharm Sci.* 2013;16(1):99-114.
2. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: The Indian solid gold. *Adv Exp Med Biol.* 2007; 595:1-75.
3. Allen RW, Schwartzman E, Baker WL, Coleman CL, Phung OJ. Cinnamon use in type 2 diabetes: an updated systematic review and meta-analysis. *Ann Fam Med.* 2013; 11(5):452-459.
4. American Diabetes Association. Available at: <http://www.diabetes.org/living-with-diabetes/treatment-and-care/medication/>. Accessed Sep 6,2015.
5. Anderson RA, Cheng N, Bryden NA, et al. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes.* 1997;46(11):1786-1791.
6. Arcaro CA, Gutierrez VO, Assis RP, et al. Piperine, a Natural Bioenhancer, Nullifies the Antidiabetic and Antioxidant Activities of Curcumin in Streptozotocin-Diabetic Rats. Essop MF, ed. *PLoS ONE.* 2014; 9(12).
7. Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. *Plant Foods Hum Nutr.* 2002 Winter; 57(1):41-52.
8. Bala K, Tripathy BC, Sharma D. Neuroprotective and anti-ageing effects of curcumin in aged rat brain regions. *Biogerontology.* 2006; 7:81–9.
9. Balamurugan AN, Akhov L, Selvarj G, Pugazhenth S. Induction of antioxidant enzymes by curcumin and its analogues in human islets: implications in transplantation. *Pancreas.* 2009;38 (4): 454-460.
10. Balon TW, Gu JL, Tokuyama Y, Jasman AP, Nadler JL. Magnesium supplementation reduces development of diabetes in a rat model of spontaneous NIDDM. *Am J Physiol* 1995; 269 (4 Pt 1): E745–52.
11. Barbagallo M, Dominguez LJ, Galioto A *et al.* Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003; **24**: 39–52.
12. Bell PM, Hadden DR. Metformin. *Endocrinol Metab Clin North Am.* 1997; 26(3):523-537.

13. Bhutani MK, Bishnoi M, Kulkani SK. Anti-depressant like effect of curcumin and its combination with piperine in unpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. *Pharmacol Biochem Behav.* 2009;92(1):39-43.
14. Bundy R, Walker A. F, Middleton R. W, Booth J. Turmeric extract may improve irritable bowel syndromesymptomology in otherwise healthy adults. A pilot study. *J Altern complement Med.* 2004; 10:1015-1018.
15. Cheng AL, Hsu, JK Lin, MM Hsu, YF Ho, TS Shen, JY Ko, JT Lin, BR Lin, W Ming-Shinag, et al. Phase I clinical trial of curcumin, a chemo preventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001:21-2895.
16. Chiasson JL, Josse RG, Gomis R, et al. STOP-NIDDM Trail Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomized trial. *Lancet.* 2002; 359:2072–7.
17. D.Suresh, K.Srinivasan. Tissue distribution and elimination of capsaicin, piperine and curcumin following oral intake in rats. *Indian J Med Res.* 2010:131-682.
18. Derosa G, Maffioli P. α -Glucosidase inhibitors and their use in clinical practice. *Archives of Medical Science: AMS.* 2012;8(5):899-906.
19. Despres JP. Potential contribution of metformin to the management of cardiovascular disease risk in patients with abdominal obesity, the metabolic syndrome and type 2 diabetes.
20. DiNicolantonio JJ, Bhutani J, O’Keefe JH. Acarbose: safe and effective for lowering postprandial hyperglycaemia and improving cardiovascular outcomes.*Open Heart.* 2015;2(1): e000327. doi:10.1136/openhrt-2015-000327.
21. Dong JY, Xun P,He K, Quin LQ. Magnesium intake and risk of type 2 diabetes: meta-analysis of prospective cohort studies. *Diabetes Care.* 2011;34(9):2116-2122.
22. Donnelly LA, Doney AS, Hattersley AT, Morris AD, Pearson ER: The effect of obesity on glycaemic response to metformin or sulphonylureas in Type 2 diabetes. *Diabet Med.* 2006, 23: 128-133.
23. El-Azab MF, Attia FM, El-Mowafy AM. Novel role of curcumin combined with bone marrow transplantation in reversing experimental diabetes: effects on pancreatic islet regeneration, oxidative stress, and inflammatory cytokines. *Eur J of Pharmacol.* 2011;658(1):41–48.

24. El-Moselhy Ma, Taye A, Sharkawi SS, El-Sisi SF, Ahmed AF. The antihyperglycemic effect of curcumin in high fat diet fed rats. Role of TNF- α and free fatty acids. *Food Chem. Toxicol.* 2011;49(5):1129-1140.
25. El-Moselhy MA, Taye A, Sharkawi SS, El-Sisi SFI, Ahmed AF. The antihyperglycemic effect of curcumin in high fat diet fed rats. Role of TNF- α and free fatty acids. *Food and Chemical Toxicology.* 2011;49(5):1129–1140.
26. Food and Nutrition Board, Institute of Medicine. Chromium. Dietary reference intakes for vitamin A, vitamin K, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C.: National Academy Press; 2001:197-223. (National Academy Press)
27. Fratiglioni L, De Ronchi D, Agüero-Torres H. Worldwide prevalence and incidence of dementia. *Drugs Aging.* 1999; 15:365–75.
28. Gaddam A, Galla C, Thummisetti S, Marikanty RK, Palanisamy UD, Rao PV. Role of Fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. *Journal of Diabetes and Metabolic Disorders.* 2015; 14:74. doi:10.1186/s40200-015-0208-4.
29. Guerrero-Romero F, Rodriguez-Moran M. Magnesium improves the beta-cell function to compensate variation of insulin sensitivity: double-blind, randomized clinical trial. *Eur J Clin Invest.* 2011;41(4):405-410.
30. Gupta A, Gupta R, Lal B. Effect of Trigonella foenum-graecum (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Assoc Physicians India.* 2001; 49:1057-1061.
31. Gupta SC, kismali G, Aggarwal BB. Curcumin, a component of turmeric: from farm to pharmacy. *Biofactors.* 2013;39(1):2-13.
32. Gupta SC, Sung B, Kim JH, Prasad S, Li S, Aggarwal BB. Multi-targeting by turmeric, the golden spice: From kitchen to clinic. *Mol Nutr Food Res.* 2013; 57(9): 1510-1528.
33. Gutierrez Vo, Pinherio CM, Assis RP, Vendramini RC, Pepato MT, Brunetti IL. Curcumin-supplemented yogurt improves physiological and biochemical markers of experimental diabetes. *Br J Nutr,* 2012; 108(3): 440 -448.
34. Habib A., Gafur A. The hypoglycaemic activity of karela fruits and fenugreek seeds in non-insulin dependent diabetic patients. *Pakistan J. Pharm.* 2003; 20:37–40.

35. Hayshi A, Suzuki H, Itoh K, Yamamoto M, Sugiyama Y. Transcription factor Nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochem. Biophys. Res. Commun.* 2003;310 (3):824-900.
36. Hiroyuki Ito, Hindenori Ishida, Yuichiro Takeuchi, Shinichi Antoku, Mariko Abe, Mizuo Mifune and Michiko Togane. Long-term effect of metformin on blood glucose control in non-obese patients with type 2 diabetes mellitus. *Nutri and Metabo* 2010; 7-83.
37. Hoehle SI, Pfeiffer E, Metzler M. Glucuronidation of curcuminoids by human microsomal and recombinant UDP-glucuronosyltransferase. *Mol. Nutr. Food Res.* 2007; 51(8): 932 -938.
38. Honda S, Aoki F, Tanaka H. et al. Effects of ingested turmeric oleoresin on glucose and lipid metabolisms in obese diabetic mice: A DNA microarray study. *J Agric Food Chem.* 2006; 54:9055–9062.
39. Iqbal, Mohammed (1993). "International trade in non-wood forest products: An overview". *FO: Misc/93/11 - Working Paper*. Food and Agriculture Organization of the United Nations.
40. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev.* 2009; 14(2):141-153.
41. Khan A, Safdar M, Khan M, Kattak K, Anderson R. Cinnamon Improves Glucose and Lipids of People with Type 2 Diabetes | *Diabetes Care.* (2003). 26(12): 3215-3218. <http://care.diabetesjournals.org/content/26/12/3215>
42. Khosla P., Gupta DD, Nagpa RK. Effect of *Trigonella foenum graecum* (Fenugreek) on blood glucose in normal and diabetic rats. *Indian J Physiol Pharmacol.* 1995;39(2):173-174.
43. Kim DC, Ku SK, Bae JS. Anticoagulant activities of curcumin and its derivative. *BMB Rep.* 2012;45(4):221-600.
44. Krasovsky J, Chang D. H. Deng G. et al. Inhibition of human dendritic cell activation by hydroethanolic but not lipophilic extracts of turmeric (*Curcuma longa*) *Planta Med.* 2009; 75:312-5.
45. Larsson, S. C. and Wolk, A. (2007), Magnesium intake and risk of type 2 diabetes: a meta-analysis. *Journal of Internal Medicine*, 262: 208–214.

46. Majithiya JB, Balarama R, Giridhar R, Yadav MR. Effect of bis[curcumino]oxovanadium complex on non-diabetic and streptozotocin-induced diabetic rats. *J Trace Elem med Biol.* 2005;18(3):211-217.
47. Mishra S, Palanivelu K. The effect of curcumin (turmeric) on Alzheimer's disease: An overview. *Annals of Indian Academy of Neurology.* 2008;11(1):13-19. doi:10.4103/0972-2327.40220.
48. Mohanty I, Arya D. S, Gupta S.K. Effect of Curcu,a longa and Ocimum sanctum on myocardial apoptosis in experimentally induced myocardial ischemic-reperfusion injuir. *BMC Complement Alter Med.* 2006; 6:3.
49. Mooren FC, Kruger K, Volker K, Golf SW, Wadeuhl M, Krasu A. Oral magnesium supplementation reduces insulin resistance in non-diabetic subjects – a double-blind, placebo-controlled, randomized trial. *Diabets Obes Meatb.* 2011;13(3):281-284.
50. Morris BW, MacNeil S, Hardisty CA, Heller S, Burgin C, Gray TA. Chromium homeostasis in patients with type II (NIDDM) diabetes. *J Trace Elem Med Biol.* 1999;13(1-2):57-61.
51. Na LX. LI Y, Pan HZ, Zhou XL, Sun DJ, Meng M, et al. Curcuminoids exert glucose-lowering effect in type 2 diabetes by decreasing serum free fatty acids: a double-blind. Placebo controlled trial. *Mol Nutr Food Res.* 2013;57(9).
52. National Center for Complementary and Integrative Health. Turmeric. Available at <https://nccih.nih.gov/health/turmeric/ata glance.htm>. Accessed September 4,2015.
53. Ng TP, Chiam PC, Lee T, Chua HC, Lim L, Kua EH. Curry consumption and cognitive function in the elderly. *Am J Epidemiol.* 2006; 164:898–906.
54. Osorio-Tobon JF, Carvalho P, Barbero GF, Nogueira GC, Rostangno MA, Meirels M. Fast analysis of curcuminoids from turmeric (*Curcuma Longa* L.) by high-performance liquid chromatography using a fused-core column. *Food Chem.* 2016; 200:167-174).
55. Pandav R, Belle SH, DeKosky ST. Apolipoprotein E polymorphism and Alzheimer's disease: The Indo-US cross-national dementia study. *Arch Neurol.* 2000; 57:824–30.
56. Paul S, jellinger. Metabolic consequences of hyperglycemia and insulin resistance. *Clin Corne.* 2007; 8:30-42.
57. Pawar RS, Toppa FA, Mandloi AS, Shaikh S. Exploring the role of curcumin containing ethanoic extract obtained from *Curcuma longa* (rhizomes) against

- retardation of wound healing process by aspirin. *Indian J Pharmacol.* 2015;47(2):160-166.
58. Peeyush KT, Giresh G, Jobin M, Paulose CS. Neuroprotective role of curcumin in the cerebellum of streptozotocin-induced diabetic rats. *Life Sci.* 2009; 85 (19-20):704-710.
 59. Platel K, Rao A, Saraswathi G, Srinivasn K. digestive stimulant action of three India spice mixes in experimental rats. *Nahrung.* 2002;46:394-398.
 60. Ponnusamy S, Zinjarde S, Bhargava S, Rajamohanan PR, Ravikumar A. Discovering Bisdemethoxycurcumin from *Curcuma longa* rhizome as a potent small molecule inhibitor of human pancreatic α -amylase, a target for type-2 diabetes.
 61. Prasad S, Aggarwal BB. Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK92752/>. Accessed September 4, 2015.
 62. Prucksusand C, Indrasukhsri B, Leethochawalit M, Hungspreugs K. Phase II clinical trial on effect of the long turmeric (*Curcuma longa* Linn.) on healing of peptic ulcer. *Southeast Asian J trop Med Public Health.* 2001; 32:208-215.
 63. SA G, Das T. Anti-cancer effects of curcumin: cycle of life and death. *Cell Div.* 2008; 3-14.
 64. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. *Clin Chim Acta* 2000; 294: 1–26.
 65. Selvi N, Sridhar MG, Swaminathan RP. Sripardha. Efficacy of turmeric as adjuvant therapy in Type 2 diabetic patients. *Ind J Biochem* 2015;30(2): 180-186.
 66. Sharma RA, Gescher AJ, Steward WP. Curcumin: The story so far. *Eur J Cancer* 2005;41(13): 1955-1968.
 67. Shimouch A, Nose K, Takaoka M, Hayashi H, Kondo T. Effect of dietary turmeric on breath hydrogen. *Dig Dis Sci.* 2008; 54(8):1725-1729.
 68. Shoba G, Joy David, Joseph Thangam, Majeed M, Rajendran R, Srinivas P. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Plant Med.* 1998; 64 (4): 353-346.
 69. Skulas-Ray AC, Kris-Etherton PM, Teeter DL, Chen CY, Vanden Heuvel JP, West SG. A high antioxidant spice blend attenuates postprandial insulin and triglyceride

- responses and increases some plasma measures of antioxidant activity in healthy, overweight men. *J Nutr*. 2011 Aug;141(8):1451-7.
70. Srinivasan A, Menon VP, Periaswamy V, Rajasekarn Kn. Protection of pancreatic β -cell by the potential antioxidant bis-o-hydroxycinnamoyl methane, analogue of natural curcuminoid in experimental diabetes. *J Pharm Pharm Sci*. 2003;6(3):327-333.
 71. Suarez A, Pulido N, Casla A, Casanova B, Arrieta FJ, Rovira A. Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats. *Diabetologia* 1995; 38: 1262–70.
 72. Tang M, Enette D, Meyer L, Liebman. Effect of cinnamon and turmeric on urinary oxalate excretion, plasma lipids, and plasma glucose in healthy subjects. *Am J Clin Nutr* 2008;87:1262-1267.
 73. Tayyem RF, Health DD, Al-Delaimy WK, Rock CL. Curcumin content of turmeric and curry powders. *Nutr Cancer*. 2006;55(2): 126-131.
 74. The Diabetes Prevention Program Research Group. Long-Term Safety, Tolerability, and Weight Loss Associated with Metformin in the Diabetes Prevention Program Outcomes Study. *Diabetes Care*. 2012;35(4):731-737. doi:10.2337/dc11-1299.
 75. Usharani P, Matten A.A, Naidu M.U.R, Raju Y.S.N., Chandra N. Effect of NCB-02, atorvastatin, and placebo on endothelia function, oxidative stress and inflammatory markers in patients with type 2 diabetes mellitus. *Drugs R.D*. 2009: 9(4): 243-250.
 76. V.Ravindrananth, N.Chandrasekhra. Absorption and tissue distribution of curcumin in rats. *Toxicology*. 1980:16-259-265.
 77. Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models. *Endocrinology*. 2008;149(7):3549–3558.
 78. Wickenberg J, Ingemansson SL, Hlebowicz J. Effects of Curcuma longa (turmeric) on postprandial plasma glucose and insulin in healthy subjects. *Nutr J*. 2010; 9:43.
 79. Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, et al. Curcuminoids enhance amyloid -beta uptake by macrophages of Alzheimer's disease patients. *J Alzheimers Dis*. 2006; 10:1–7.

APPENDIX A

SAMPLE SIZE CALCULATIONS

SAMPLE SIZE CALCULATIONS

Power calculations were conducted online using the software developed by David Schoenfeld with support from the MGH Mallinckrodt General Clinical Research JavaScript version developed by REMorse.

Study by Wickenburg J et al. regarding turmeric ingestion and its effects on postprandial on insulin levels was examined. The minimal detectable difference in means was calculated as 254. Within patient standard deviation was calculated based on provided SEM (Standard Error Mean) and sample size n. This calculation gave 374. Hence, with 81% probability that the study will detect a treatment difference in order to see a significant change at a two-sided 0.05 significant level in insulin levels at 30 min postprandial, a total sample size of 37 patients was calculated.

Our goal was to recruit 15 participants for this two-treatment cross over study, as to follow Wickenburg J et al. study. However, we were short in achieving that number as our sample size for our study was 8.

APPENDIX B

INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL

APPROVAL: MODIFICATION

Carol
Johnston
SNHP:
Nutrition
602/827-
2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 10/28/2015 the ASU IRB reviewed the following protocol:

Type of Review:	Modification
Title:	Effects of Curcuma longa (turmeric) on postprandial glycemia, blood lipid profile, and bowel transit time.
Investigator:	Carol Johnston
IRB ID:	STUDY00003267
Funding:	Name: Graduate College
Grant Title:	None
Grant ID:	None
Documents Reviewed:	<ul style="list-style-type: none"> • protocol, Category: IRB Protocol; • reference, Category: Technical materials/diagrams; • online survey, Category: Recruitment Materials; • instructions, Category: Participant materials (specific directions for them); • bowel activity sheet, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • flyer and email text, Category: Recruitment Materials; • adherence calendar, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • consent , Category: Consent Form; • health history questionnaire, Category: Screening forms;

The IRB approved the modification.

When consent is appropriate, you must use final, watermarked versions available under the “Documents” tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

APPENDIX C
CONSENT FORM

EFFECTS OF HERBAL SUPPLEMENT ON POSTPRANDIAL GLYCEMIA,

INTRODUCTION

BLOOD CHOLESTEROL AND BOWEL TRANSIT TIME

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

RESEARCHERS

Dr. Carol Johnston, a Nutrition professor at Arizona State University Downtown Campus has requested your participation in a research study.

STUDY PURPOSE

The purpose of the research is to examine the effects of a common herb for improving common health parameters including blood glucose and cholesterol concentrations and bowel regularity.

DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are 18 years of age or older, a non-smoker and healthy but experience some degree of bowel irregularity. Participants will be asked to maintain their usual diet and physical activity level throughout the trial with the exception of the day prior to meal testing. This study will initially involve the completion of a brief health history questionnaire to demonstrate the absence of medical conditions that may impact the study. Your weight, height, and girth will be measured at this time. This first meeting will take about 15 minutes. There are four additional visits: 2 meal test visits that will last about 2 hours and 2 short visits scheduled three weeks after meal testing. The procedures on meal test days are identical. On the day prior to meal testing you are asked to avoid heavy exercise (normal activities such as walking to work or walking the dog is ok). You will be asked to eat a normal breakfast and lunch of your choice. For the evening meal, we will ask you to eat chicken, turkey, fish, or eggs with bread or rice. We will also provide a bagel for you to eat with this meal. Following dinner, you will fast overnight and not consume any food or beverage with the exception of water. Participants will be asked to brush teeth thoroughly the night before the test and the morning of the test and to floss between teeth thoroughly the night before the test and the morning of the test. The following morning, you will travel to ASU (the Nutrition labs at the ABC1 Building on the ASU Downtown campus) early in the morning. We will collect a blood sample from a vein, and your finger will be pricked for another blood sample. You will sit down and consume a test meal (mashed potatoes and juice). Your finger will be pricked four more times over the next 2 hours. You may drink water during these two hours but you are not to consume any food and you cannot lie down. We will collect another blood sample from a vein at 30 minutes postmeal, and

we will ask you to breath into a valve prior to eating the test meal and at 60 and 120 minutes postmeal. During this 2-hour test period, you may read, study, or work on the computer at the test site. Once testing is complete, you may proceed with your normal activities. You will be provided with capsules to take daily for the next 3 weeks. We will also ask you to complete a daily report to assess bowel function. At the end of the 3 weeks, we ask you to return to the test site in a fasted state to provide a venous blood sample, a single breath test, and a single finger prick, and to turn in your bowel activity sheets. This entire process will be repeated after a 5 to 6-week interval.

Blood sampling and finger pricks will be conducted under sterile conditions using disposable, retractable lancets, and the level of glucose and cholesterol in blood will be recorded. Your breath will be analyzed for hydrogen.

RISKS

Bruising of the skin or a feeling of faintness is possible during the finger pricks and blood draws. Disposable retractable lancets will be used and sterile conditions will be used. The herbal supplement being tested is commonly used around the world and no adverse effects are anticipated. However, if any stomach or intestinal concern develops during the study, such as diarrhea or constipation, please contact the investigators.

BENEFITS

There is no direct benefit for participating in this trial. If desired, you will be provided with study results and your personal blood data at the end of the study.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

WITHDRAWAL PRIVILEGE

You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not incur negative treatment to you by the researchers.

COSTS AND PAYMENTS

The all test foods and supplements will be given to you during the study free of charge. You will receive four Target cards [one card at visits 2 (\$5), 3 (\$10), 4 (\$15), and 5 (\$20)] for a total of \$50. You may need to pay for parking during your visits.

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form, you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

Subject's Signature Printed Name Date _____

Contact phone number Email _____

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator _____ Date _____

APPENDIX D

TEST MEAL INGREDIENTS

Mashed Potato Ingredients

Serves 1 (~300 grams)

Ingredients

- 400 g *Russet Potatoes*, peeled and cut lengthwise into quarters
- 1/4 (1.5 grams) teaspoon salt
- 2 Tbsp (15 grams) heavy cream
- 1 Tbsp (15 g) butter
- 1 Tbsp milk (or more)
- Salt 1/4 teaspoon and Pepper 1/2 teaspoon

APPENDIX E

TEST MEAL RECIPE

Mashed Potato Recipe – Method

- 1) Start 4 cups of water to boil in a deep saucepan.
- 2) Add one teaspoon of salt to the water.
- 3) Place the peeled and cut potatoes into a medium saucepan. **Wg: 2721 grams**
- 4) Turn the heat on to high, and bring the water to a boil. **Time: 15 minutes**
- 5) Reduce the heat to low to maintain a simmer, and cover. **Time: 10 minutes**
- 6) Cook for 15 to 20 minutes, or until you can easily poke through them with a fork.
- 7) While the potatoes are cooking, melt the butter and warm the cream. You can heat them together in a pan on the stove or in the microwave.

Time → Melt the butter 8 Tbs. (113 g) for 30 sec and mix it with cream 160 g and warm for 10 seconds microwave

- 8) When the potatoes are done, drain the water and place the steaming hot potatoes into a large bowl.
- 9) Pour the heated cream and melted butter over the potatoes. Mash the potatoes with a potato masher.

Time for mashing potatoes: 45 mashes

- 10) Then use a strong wooden spoon (a metal spoon might bend) to beat further.

Time for beating the potatoes: one-min

- 11) Add milk and *beat until the mashed potatoes are smooth.

Milk: 112 g Time to beat: ~ 2 min

*Don't over-beat the potatoes or the mashed potatoes will end up gluey.

- 12) Add salt and pepper to taste.